JOURNAL OF AGRICULTURAL RESEARCH

VOL. 58 WASHINGTON, D. C., APRIL 1, 1939 NO. 7

THE FOLIAR DIAGNOSIS OF ZEA MAYS SUBJECTED TO DIFFERENTIAL FERTILIZER TREATMENT [1]

By WALTER THOMAS, *professor of plant nutrition*, and WARREN B. MACK, *professor of vegetable gardening, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

In the method of foliar diagnosis (*1, 5*)[2] the development and yield of a plant is related to the series of chemical transformations taking place in the synthetic laboratory of the plant, viz the leaf. The experimental facts established by the method are proof that this basis of reference is more reliable and in addition, supplies much more information concerning the factors governing the development and yield of a plant than does the traditional method of relating ultimate yields solely to the fertilizers applied. Since it requires only the collection of a few leaves it is not only simpler and more practical, but is a much more sensitive method of control than that requiring the analysis of the entire plant (*3*).

Foliar diagnosis makes it possible to study the variations in the mineral nutrition of the plant resulting from very diverse factors—physical, chemical, and biological. The evidence obtained thus far (*1, 5, 6, 7*) indicates that the composition of a leaf is modified both quantitatively and qualitatively with respect to nitrogen, phosphoric acid, and potash by factors hitherto little suspected.

There are valid grounds for believing that sense can be made of the chaotic mass of information in agronomical literature by the application of the method to problems of fertilizer effects that now appear paradoxical.

The experimental facts upon which the validity of the method is based consist in essence of the following findings:

(1) Two morphologically homologous leaves of the same species and variety are the seat of identical physiological processes when the medium (soil and meteorological factors) is identical, and of different physiological processes when the mediums are different (*1, 5*).

(2) Whenever a fertilizer element (whether N or P or K) is effective, as determined by response to that element, the response is invariably associated with an increase of that element in the dried material of the leaf (*1, 5, 6, 7, 8*).

(3) The foliar diagnoses of plants of the same species growing on the same homogenous medium (soil and meteorological factors) but receiving different fertilizer treatments are correlated with their development and also with the nature of the fertilizer applied (*1, 7*).

It is emphasized that the results by the method of foliar diagnosis are comparative just as the results of the entire analysis of plants are comparative (*3*). No physiological significance, therefore, can

[1] Received for publication July 18, 1938. Paper No. 843 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 491.

be attached to the foliar diagnosis of any one fertilizer treatment (plot) considered alone.

The results are expressed on the only suitable basis for this type of investigation, viz, on the basis of the dry weight of the leaves. All results by means of which the method has been established are on the basis of the percentage of the element in the dried foliage.

In the investigations on foliar diagnosis carried out by the present writers thus far the influence of calcium and magnesium on the N-P-K nutrition has not been examined, although the desirability for such knowledge has been pointed out (*8*). Since the foliar diagnosis, with respect to Ca, of plants sprayed with calcium-containing sprays would be worthless, *Zea mays* growing on certain plots of the Jordan fertility experiment of the Pennsylvania Agricultural Experiment Station was used for the present studies. An unfertilized plot, not available in the earlier studies of the method, was also included. This paper reports the results of these studies.

Ranking in age second only to the Rothamsted experimental plots, the Jordan plots laid out in 1881 afford interesting experimental material for the development of laws, principles, or axioms of plant nutrition under field conditions.

In long-continued field experiments in which a given plot receives year after year the same fertilizer, any disequilibrium becomes progressively greater and greater with time. Trials of a few years only may frequently not show this disequilibrium.

MATERIALS AND METHODS

The Jordan fertility plots (*2*) consist of 144 plots of one-eighth acre each, arranged in four tiers consisting of 36 plots. On each tier a 4-year rotation is followed in which corn, oats, wheat, and hay (mixed clover and timothy) are each grown for 1 year, the fertilizer treatments being applied to the corn and the wheat.

A detailed description of the method of leaf sampling was given in an earlier publication (*5*). The third leaf from the base of a stalk of corn was taken on July 6, July 21, August 8, and August 25, 1936, from all of the plants along a row lengthwise of each plot, omitting plants that deviated considerably from the norm. Successive rows were then sampled on each of the sampling dates indicated.

The plot numbers, treatments, and yields of the plots examined are given in table 1.

TABLE 1.—*Plot, treatments, and yields in tier 1*

Plot No.	Treatment	N, P_2O_5, and K_2O equivalent applied to each plot	Symbol and ratio	Yield per plot	
				Grain	Stover
		Pounds		*Pounds*	*Pounds*
1	Nothing	0		165.4	117.1
2	Dried blood	3	N	265.6	143.4
3	Superphosphate	6	P	371.2	220.1
4	Muriate of potash	12.5	K	298.4	181.7
5	Dried blood Superphosphate	3 6	NP, 6:12:0	376.7	195.9
6	Dried blood Muriate of potash	3 12.5	NK, 6:0:25	263.5	171.7
7	Superphosphate Muriate of potash	6 12.5	PK, 0:6:25	497.4	254.5
9	Dried blood Superphosphate Muriate of potash	3 6 12.5	NPK, 6:12:25	520.2	214.0

METEOROLOGICAL CONDITIONS DURING THE 1936 GROWING SEASON

As a matter of record the meteorological conditions for the month preceding the sampling, and for the intervals between sampling are given in table 2.

TABLE 2.—*Rainfall and average daily mean temperatures during the month preceding sampling and during the intervals between sampling*

Period	Rainfall	Mean daily temperature	Period	Rainfall	Mean daily temperature
	Inches	*° F.*		*Inches*	*° F.*
June 6–July 5	5.04	66.7	July 22–Aug. 8	3.38	70.6
July 6–July 21	.51	75.3	Aug. 9–Aug. 25	2.48	74.4

PRESENTATION OF RESULTS

The results of the analysis of the third leaf from the base are shown in table 3 expressed as (1) percentages of nitrogen, phosphoric acid, potash, lime, and magnesia in the dried foliage, (2) milligram equivalents, and (3) the composition of the NPK unit (*5*).

THE DERIVATION OF THE NPK UNIT

Two concepts, viz, that of quantity and that of quality of the mineral constituents are inherent in the expression of the analysis in terms of the dried weight of the leaves. The quantity or intensity of nutrition of the selected leaves consists of the sum ($N+P_2O_5+K_2O$) of the dominant fertilizer entities at the moment of sampling, the data for which are shown in column 7. The quality of nutrition is the ratio of these entities to each other also at the moment of sampling. Since this factor is dependent upon and related to the chemical reactions involved in the leaf, it is necessarily expressed in milligram-equivalent values.

Let M_x, M_y, M_z be the percentages of nitrogen, phosphoric acid, and potash in the leaf at the moment of sampling. Then, expressing in milligram-equivalents:

$$E_x=1{,}000\frac{M_x}{N};\ E_y=1{,}000\frac{M_y}{\frac{1}{6}(P_2O_5)};\ E_z=1{,}000\frac{M_z}{\frac{1}{2}(K_2O)}$$

Putting $S=E_x+E_y+E_z$ and dividing by S, we get $1=\frac{E_x}{S}+\frac{E_y}{S}+\frac{E_z}{S}$. The last three terms represent respectively in milligram-equivalent values the proportional parts of N, P_2O_5, and K_2O in a unit quantity of these elements. To avoid fractional values the terms are multiplied by 100. If the respective values obtained be denoted by X, Y, Z then $X+Y+Z=100$. This is the NPK unit shown in the last three columns of table 3.

TABLE 3.—*The percentages of N, P_2O_5, K_2O, CaO, and MgO in the dried foliage, milligram equivalents, and the composition of the NPK unit for plants grown under different fertilizer treatment in tier 1*

Date	Mineral content of dried foliage						Milligram equivalents				Composition of the NPK unit		
	N— (M_x)	P_2O_5— (M_y)	K_2O— (M_z)	CaO—	MgO—	N+P_2O_5+K_2O— $M_x+M_y+M_z$ (s)	N— (E_x)	P_2O_5— (E_y)	K_2O— (E_z)	$E_x+E_y+E_z$— S	X— $\left(100\frac{E_x}{S}\right)$	Y— $\left(100\frac{E_y}{S}\right)$	Z— $\left(100\frac{E_z}{S}\right)$
NOTHING (PLOT NO. 1)													
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*							
July 6	2.960	0.307	2.387	2.308	0.934	5.654	211.344	12.986	50.843	275.173	76.804	4.719	18.477
July 21	2.210	.305	2.515	2.087	.936	5.030	157.794	12.901	53.569	224.264	70.361	5.753	23.886
Aug. 8	1.800	.230	1.841	1.605	1.039	3.871	128.520	9.729	39.213	177.462	72.421	5.482	22.097
Aug. 25	2.010	.316	1.608	1.999	1.216	3.934	143.514	13.367	34.250	191.131	75.089	6.993	17.920
N (PLOT NO. 2)													
July 6	3.310	0.311	2.248	2.058	1.177	5.809	236.334	13.155	47.882	297.371	79.474	4.424	16.102
July 21	2.420	.330	2.104	2.435	1.448	4.854	172.788	13.959	44.815	231.562	74.618	6.028	19.353
Aug. 8	2.090	.260	1.453	2.328	1.376	3.803	149.226	10.998	30.949	191.173	78.059	5.753	16.189
Aug. 25	2.340	.348	1.481	2.222	1.159	4.169	167.076	14.720	31.545	213.341	78.314	6.899	14.786
P (PLOT NO. 3)													
July 6	3.210	0.656	1.019	3.131	1.901	4.888	229.194	27.749	21.705	278.648	82.252	9.958	7.789
July 21	2.260	.841	.756	3.744	2.462	3.857	161.364	35.574	16.103	213.041	75.743	16.698	7.559
Aug. 8	2.060	.602	.794	3.348	1.847	3.456	147.084	25.465	16.912	189.461	77.633	13.441	8.926
Aug. 25	2.100	.678	.911	2.937	1.792	3.689	149.940	28.679	19.404	198.023	75.718	14.483	9.799
K (PLOT NO. 4)													
July 6	2.980	0.301	3.984	1.690	0.815	7.265	212.772	12.732	84.859	310.363	68.556	4.102	27.342
July 21	2.280	.263	4.000	1.887	1.112	6.543	162.792	11.125	85.200	259.117	62.826	4.293	32.881
Aug. 8	2.320	.270	3.901	1.689	.905	6.491	165.648	11.421	83.091	260.160	63.672	4.390	31.938
Aug. 25	2.020	.392	2.480	1.491	.742	4.892	144.228	16.582	52.824	213.634	67.512	7.762	24.726

NP (PLOT NO. 5)

July 6	3.210	0.928	0.724	3.410	2.114	4.862	229.194	39.254	15.421	283.869	80.739	13.828	5.432
July 21	2.410	.692	.813	3.322	2.516	3.915	172.074	29.272	17.317	218.663	78.694	13.387	7.919
Aug. 8	2.360	.690	.813	3.322	2.027	3.863	168.504	29.187	17.317	215.008	78.391	13.575	8.054
Aug. 25	2.370	.768	.775	2.984	2.281	3.913	169.218	32.486	16.507	218.211	77.548	14.887	7.565

NK (PLOT NO. 6)

July 6	3.040	0.336	4.031	1.543	0.634	7.407	217.056	14.213	85.860	317.129	68.444	4.482	27.074
July 21	2.390	.280	4.209	1.507	.833	6.879	170.646	11.844	89.652	272.142	62.705	4.352	32.943
Aug. 8	2.000	.262	3.081	1.628	.905	5.343	142.800	11.083	65.025	219.508	65.055	5.049	29.896
Aug. 25	2.380	.376	2.763	1.309	.526	5.519	169.932	15.905	58.852	244.689	69.448	6.500	24.052

PK (PLOT NO. 7)

July 6	3.040	0.440	3.565	1.902	0.605	7.045	217.056	18.612	75.934	311.602	69.658	5.973	24.369
July 21	2.280	.476	3.508	2.221	.869	6.264	162.792	26.135	74.720	257.647	63.184	7.815	29.001
Aug. 8	1.960	.420	2.887	2.283	.688	5.267	139.944	17.766	61.493	219.203	63.842	8.105	28.053
Aug. 25	2.080	.534	2.665	1.917	.507	5.269	148.512	22.588	56.551	227.651	65.237	9.922	24.841

NPK (PLOT NO. 9)

July 6	3.03	0.438	3.674	1.720	0.606	7.506	216.342	18.527	78.256	313.125	69.091	5.917	24.992
July 21	2.460	.454	3.766	1.887	.707	6.680	175.644	19.204	80.216	375.064	63.856	6.982	29.163
Aug. 8	2.620	.564	2.992	1.842	.362	6.176	187.068	23.857	63.729	274.654	68.110	8.686	23.203
Aug. 25	2.540	.626	2.879	1.705	.271	6.045	181.356	26.479	61.323	269.158	67.379	9.838	22.783

DISCUSSION AND INTERPRETATION OF RESULTS

In the discussion which follows, the treatment symbol followed by the number of the plot is a brief method of expressing the content of N, P_2O_5, or K_2O in the third leaf of plants growing on the particular plot with the treatment represented. For example, "N in NP (No. 5)" means the content of N in the third leaves of plants growing on the plot fertilized with NP, which is plot No. 5.

NITROGEN

At the first sampling the differences in the nitrogen content of the leaves of plants growing on check (No. 1), K(No. 4), NK(No. 6), PK(No. 7), and NPK(No. 9) plots were small (0.08 percent or less), with no difference between those of P(No. 3) and NP(No. 5). At this date the content of N was highest in N(No. 2), followed by P(No. 3) and NP(No. 5).

With advancing age, the leaves from the plots receiving nitrogen became more and more differentiated, in their N content, from those on plots receiving none. This difference shows that the nitrogen of dried blood, slowly available during the first period, was now becoming more available.

The nitrogen content did not decrease regularly with the advancing age of the leaves of plants growing on any of the plots considered in this paper. Accumulation of nitrogen took place alike in plots treated with nitrogen as commercial fertilizer (dried blood) and in those not receiving nitrogen. Thus accumulation took place in the last period in check, N, P, NK, and PK and during the second period in K and NPK, showing slow and poor utilization of nitrogen even by plants which received nitrogen applications in the form of dried blood and which had never received lime amendments.

At two sampling dates only, July 21 and August 25, are the leaves of plants growing on plots that received nitrogen applications all higher in nitrogen than those on plants that did not receive any. At these dates there is a relationship, consequently, between the content of nitrogen in the leaf and in the fertilizer, but none between nitrogen in the leaf and the yield, nor between the presence of nitrogen in the fertilizer and the yield, except when accompanied by phosphoric acid and potash.

Graphed in the manner previously described (*4*), marked disequilibrium between nitrogen and phosphoric acid of the leaves of plants from all treatments is shown (fig. 1). At the first sampling date the phosphoric acid content of the leaf is too low in relation to the nitrogen in all treatments except P and NP. In the latter and also in PK after the first period, the nitrogen is too low in relation to the phosphoric acid throughout the whole period from July 6 to August 25. After July 21, no uniformity in the relations between nitrogen and phosphoric acid of the leaf is shown. Nitrogen is much too high in relation to phosphoric acid in both check and N throughout the whole period and also in K up to August 8, when the relationship is reversed. In NPK the tendency is toward nutritional equilibrium between N and P_2O_5 up to the beginning of the third period, after which the nitrogen becomes too low in relation to the P_2O_5.

PHOSPHORIC ACID

Phosphoric acid does not decrease progressively with the advancing age of the leaf. In the leaves of check, N, K, NK, PK, and NPK a maximum occurs at the last sampling, whereas in P and NP the maximum occurs on July 21 and July 6, respectively.

The differences in the foliar diagnosis caused by the presence of phosphoric acid in the fertilizer, although small absolutely, are very

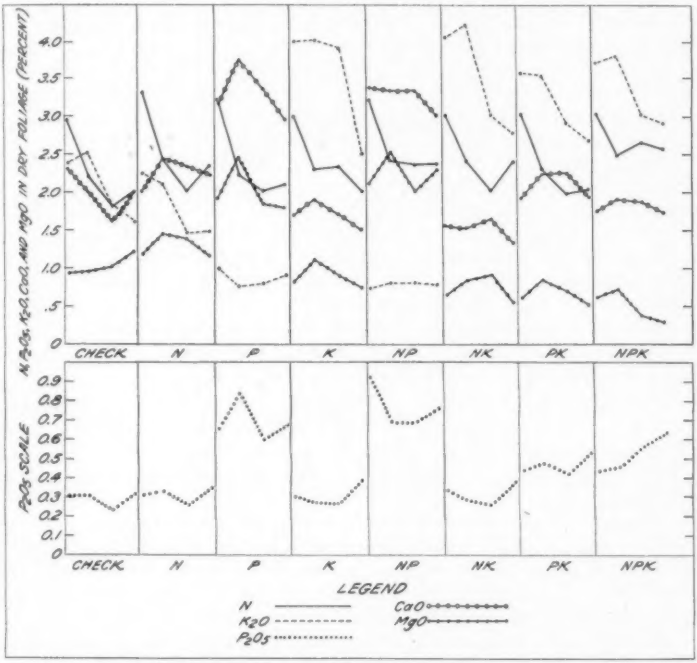

FIGURE 1.—Changes during the growth cycle in the content of N, P_2O_5, K_2O, CaO, and MgO in the third leaf resulting from different treatments. Percentage values in terms of the dried foliage as ordinates and dates of sampling as abscissae.

striking relatively. Thus a variation from 0.3 percent in K to 0.6 percent in P is a variation of 100 percent.

Phosphoric acid is very much higher throughout the whole period in the leaves of plants which received superphosphate additions. Thus, on July 6 the phosphoric acid content of the selected leaves from plots which received superphosphate applications is 0.438 percent or more; whereas in those which did not receive phosphate fertilizer the content of P_2O_5 is 0.336 percent or less.

Furthermore, the leaf content with respect to P_2O_5 is highest throughout the whole period in the four plots which have the highest yields—P, NP, PK, and NPK. But the highest yield does not correspond to the P_2O_5 graph which is the highest—viz, the NP, but to NPK in which the P_2O_5 graph is the next to the lowest of these four highest yielding plots. Accordingly, the additional supplement of P_2O_5 absorbed by

the leaves from P and NP has not been utilized in growth—an example of "luxuskonsumption" of phosphorus.

A relationship, therefore, exists between phosphoric acid in the leaf and phosphoric acid in the fertilizer, between phosphoric acid in the leaf and the yield, and between phosphoric acid in the fertilizer and the yield.

POTASH

The potash content of the leaves of plants growing on the plots that received potash fertilizer is very much higher than in those that did not. At the first sampling, on July 6, the content of K_2O was 3.56 percent or more in the leaves of plants that received potash applications and only 2.38 percent or less in those that did not.

The K_2O graphs of P and NP, two of the four highest yielding plots, are lower than any other of the treatments, the causes for which will be considered later. Furthermore, the leaf content in K_2O of NK, the second lowest yielding plot, is on the first two sampling dates the highest of any of the treatments.

There is consequently no relationship between the presence of potash in the fertilizer and the content of potash in the leaf, and there is no relationship between the variation, however great, of the potash in the leaf and the variations in the yield. There is, moreover, no relationship between the presence of potash in the fertilizer and the yield; thus, potash increased the yields when applied alone or with phosphate, but not when applied with nitrogen.

LIME AND MAGNESIA

With the exception of NP, the changes in direction of the graphs for MgO with lapse of time are similar to those of the respective graphs for CaO.

In leaves of plants developing normally a progressive increase occurs in the CaO and MgO content of the leaf with advancing age. But in check, P, K, NP, NK, and NPK, the CaO content was lower at the last sampling on August 25 than at the first sampling on July 6 and, moreover, the increase in CaO with the advancing age of the leaf was very small in the remaining treatments—N and PK. It should be kept in mind that none of these plots has been limed since 1881.

The effect of superphosphate with and without potash applications on the absorption of calcium and magnesia is strikingly shown in table 4, in which the results for CaO and MgO are given as the mean of the results in each case of the four samplings expressed as a percentage of the dried foliage.

TABLE 4.—*The effect of superphosphate with and without muriate of potash on the absorption of calcium and magnesium*

Plot No.	Treatment	CaO	MgO	Plot No.	Treatment	CaO	MgO
		Percent	*Percent*			*Percent*	*Percent*
3	P	3.29	2.00	2	N	2.26	1.29
5	NP	3.26	2.23	4	K	1.69	.89
7	PK	2.08	.66	7	NK	1.50	.72
9	NPK	1.79	.49	23	CaO	3.55	1.18
1	Nothing	2.00	1.03				

The CaO and MgO contents of the leaves of P and NP, which received superphosphate without potash additions, are relatively high; the CaO content is nearly as high as in the leaves from the plot which received dressings of lime only without fertilizer, whereas the MgO content is actually higher in P and NP than in the lime plot. On the other hand, in PK and NPK, which received both superphosphate and potash applications, the CaO and MgO contents of the leaves are very much lower. Confirmation of this depressing effect of muriate of potash on the absorption of CaO and MgO is shown by a comparison of the results of the CaO and MgO contents of the leaves of K and NK with those of check and N.

Nevertheless, the reduction in the CaO and MgO content of the leaves of NPK and PK below the values in P and NP has had no adverse effect on the yields of NPK and PK. It would appear, consequently, that calcium and magnesium tend to replace potassium when this element is deficient. The functions of potassium in which it may be replaced by calcium and magnesium can be only conjectured, but are probably connected with controlling the hydrogen-ion concentration of the sap.

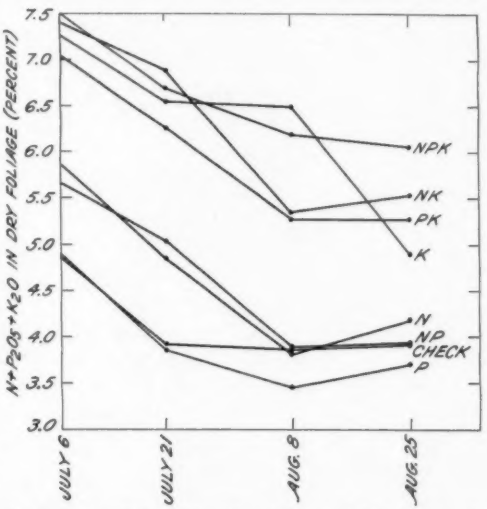

FIGURE 2.—Intensities of nutrition of the leaves at four dates of sampling.

THE INTENSITIES OF NUTRITION

The magnitudes representing the intensities of nutrition (*5*) of the chosen leaves on the respective sampling dates are given in column 7, table 2, and are shown graphically in figure 2.

Two groups are recognized, check, N, P, and NP, having low intensities of nutrition, and K, NK, PK, and NPK, with relatively high intensities of nutrition. The addition of potash, then, invariably resulted in an increase in the intensity of nutrition of the leaf as compared with that of those which did not receive potash treatment; but, as already pointed out (*5*), there is not necessarily any relation between intensity taken alone and the yields. A fertilizer may intervene to cause (1) a change in the intensity or (2) a change in the NPK equilibrium, or a change in (1) and (2) simultaneously. It is to be noted that NPK has the highest mean intensity and the highest yield of the plots here considered. The intensities resulting from the other treatments must consequently be regarded as below the optimum in satisfying the mineral requirements with respect to N, P, and K.

COMPOSITION OF THE NPK UNITS

The composition of the NPK units at the respective sampling dates is shown in the last three columns of table 3. The diagrammatic representation of this unit has been given previously (*5*). The NPK unit represents the equilibrium between nitrogen, phosphoric acid, and potash in the chosen leaves at the moment of sampling.

It has been shown (*5*) that this unit is independent of the intensity factor of nutrition ((*s*) table 3) and consequently is a magnitude representing the quality factor of nutrition as distinguished from the

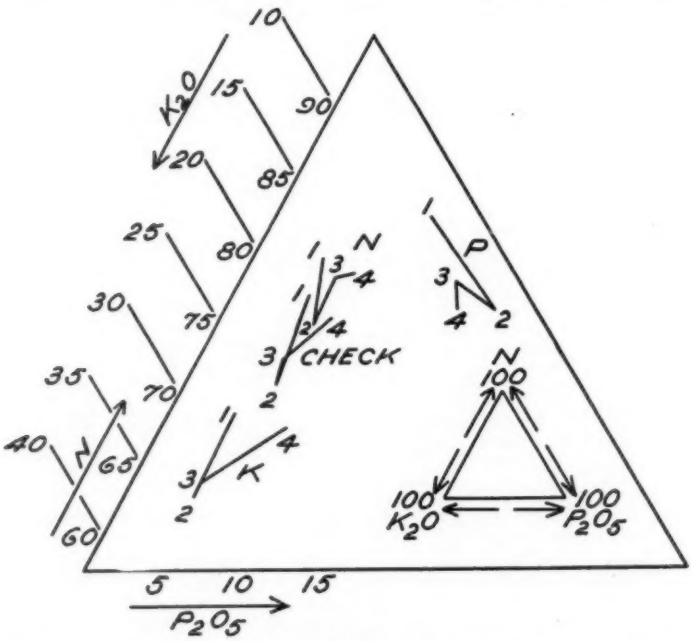

FIGURE 3.—Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium in the third leaf from plants growing on N, P, K, and nothing (check) plots. The numerals indicate the coordinate point of the NPK unit at the respective sampling date.

intensity or quantity factor. These two mangitudes representing the quality and quantity factors, respectively, in the selected leaf although abstractly distinct are inseparably connected with the physiological processes of the leaf and are, therefore, to be considered simultaneously.

It must be borne in mind further that the effect of a fertilizer is the resultant of (1) the effect of the fertilizer itself, and (2) of the medium (soil and meteorological factors) on the N–P_2O_5–K_2O equilibrium.

THE COURSE OF NUTRITION WITH RESPECT TO NPK

The detailed diagrams showing the course of nutrition with respect to NPK during the period of sampling are given in figures 3, 4, and 5. The points represented by the numerals are the coordinate points at

the four successive samplings. It is not necessary to discuss these graphs in detail, for the eye can follow the course of nutrition resulting from the different treatments by following the lines from one sampling date to the next.

The reader should keep in mind that the changes in the composition of the NPK unit of any treatment with time corresponds with the changes in the N–P_2O_5–K_2O equilibrium with the advancing age of

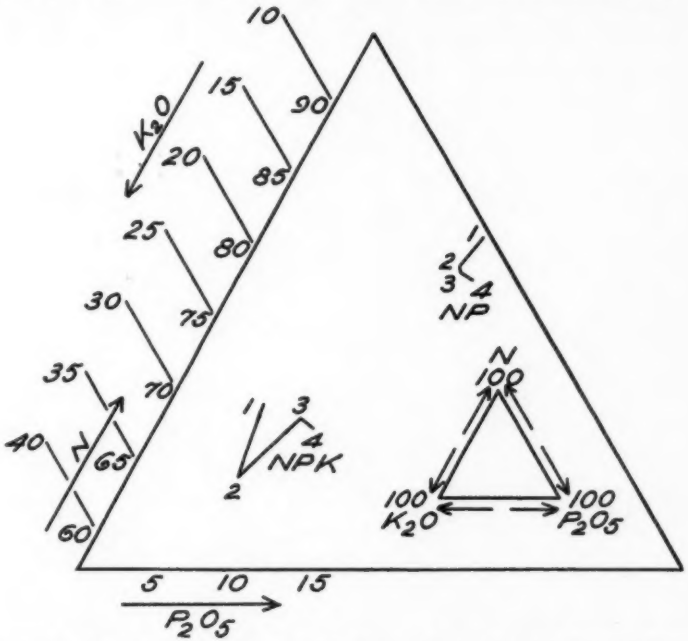

FIGURE 4.—Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium in the third leaf from plants growing on the NP and NPK plots. The numerals indicate the coordinate point of the NPK unit at the respective dates of sampling.

the leaf (*5*) as the result of the influence of the medium (soil and climate).

The graphs show the following characteristics: Throughout the earlier period (1–2) potash increases at the expense of the nitrogen, and in the two treatments (NP, NK) at the expense of the P_2O_5 also During the remainder of the growth period (2–4), nitrogen increases chiefly at the expense of the potash, except in two treatments (P and NP).

These facts reflect the slow availability of dried blood on unlimed plots. Comparison of the graphs of the treated plots with that of the check plot shows that the addition of nitrogen in the form of dried blood has been unable to overcome, during the earlier period, the declining tendency of the medium (soil and meterological factors) with respect to nitrogen supply. The reversal in direction after

July 21 of the graph for the check plot shows that the unfavorable influence of the medium with respect to nitrogen supply is arrested and becomes more favorable as the season progresses.

The Mean NPK Unit

The center of gravity of each of these diagrams represents the mean NPK unit of the leaf of the rank selected. The mean NPK units of the leaves of plants growing on the respective plots, therefore, can be indicated on the triangle by means of a point, as shown in figure 6.

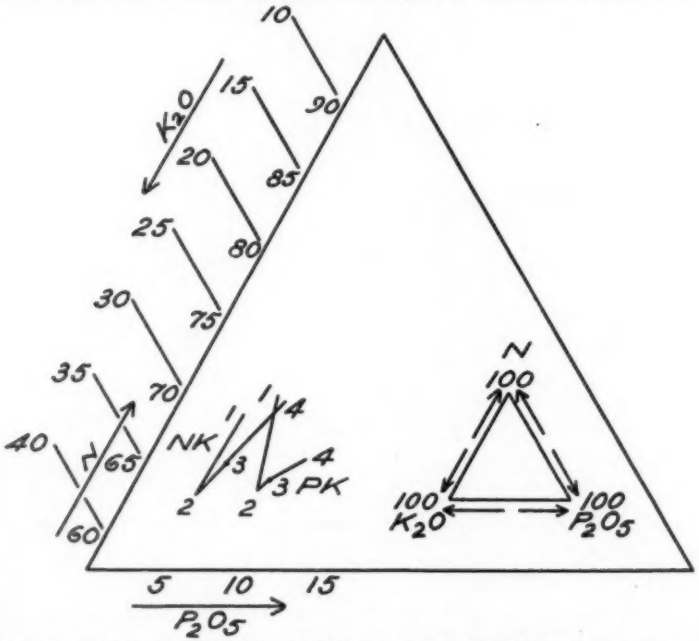

FIGURE 5.—Changes during the growth cycle in the N–P_2O_5–K_2O equilibrium in the third leaf from plants growing on the NK and PK plots. The numerals indicate the coordinate point of the NPK unit at the respective dates of sampling.

Table 5 lists the mean NPK units and the mean intensity of nutrition together with the yields of grain and stover.

TABLE 5.—*The relation of the composition of the NPK unit and intensity to yields*

Group No.	Plot No.	Treatment	Mean intensity of nutrition	Mean NPK-unit	Yields per plot	
					Grain	Stover
					Pounds	*Pounds*
1	1	Check	4.62	73.67:5.74:20.59	165.6	117.1
	6	NK	6.29	66.41:5.10:28.49	263.5	171.7
	2	N	4.67	77.62:5.77:16.61	265.6	143.4
	4	K	6.29	66.64:5.14:29.22	298.4	181.7
9	3	P	3.97	77.84:13.64:8.52	371.2	220.1
	5	NP	4.14	78.84:13.92:7.24	367.7	195.9
3	7	PK	5.96	65.48:7.95:26.57	497.4	254.5
	9	NPK	6.60	67.11:7.85:25.03	520.2	214.0

THE CLASSIFICATION INTO THREE GROUPS ACCORDING TO THE POSITION ON THE TRIANGLE OF THE MEAN NPK UNITS

The treatments fall into three groups according to their position on the triangle.

Group 1: Check, N, K, and NK. Poorly nourished plants having the lowest yields from any of the plots, and characterized by a position on the triangle further to the left—i. e., further removed from the apex representing P_2O_5=100 percent.

Group 2: P and NP. Better nourished, with yields higher than those

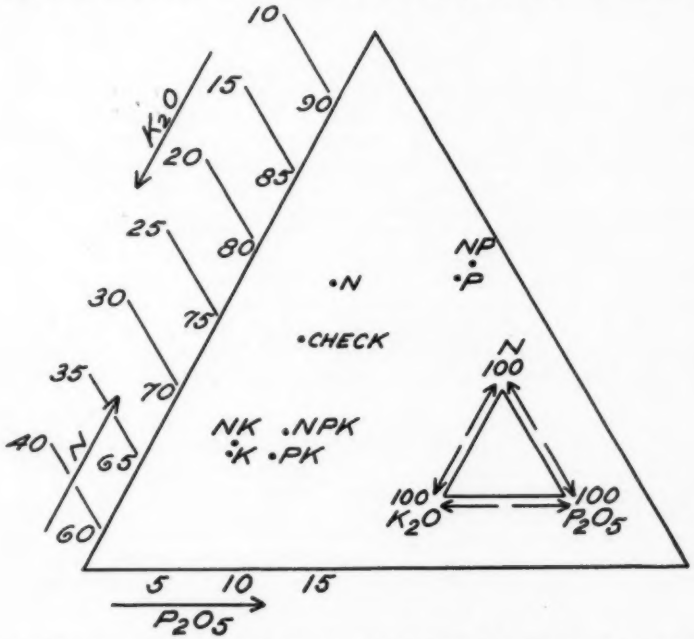

FIGURE 6.—Relative positions of the mean NPK units of the third leaf from plants growing on the plots indicated. The mean NPK unit represents the center of gravity of the respective graphs of figures 3 to 5.

of group 1 and characterized by a position further to the right—toward the apex representing P_2O_5=100 percent, and relatively high up toward the apex N=100 percent.

Group 3: NPK and PK. Much better nourished plants having the highest yields from any of the plots considered in this paper. These two plots are characterized by a position further to the right of the lowest yielding group 1, and to the left of the intermediate yielding group 2—i. e., the leaves of plants in this group (3) contain more P_2O_5 in the NPK unit than do those of group 1, and also more K_2O but less P_2O_5 than those of group 2.

As already stated (p. 487) the quality of nutrition must be considered simultaneously with the quantity of nutrition—the intensity factor. In group 1 are to be found plants having relatively low in-

tensities (check, and N) on the one hand, and on the other, those possessing relatively high intensities (K and NK), which are the results, respectively, of a very low and of a relatively high content of K_2O in the NKP unit. Plots in group 2 are characterized by very low intensities of nutrition and those in group 3, the high-yielding group, by high intensities.

SUMMARY

Plants of *Zea mays* growing on unlimed experimental plots treated with different combinations of commercial fertilizers were examined by the method of foliar diagnosis. The plots reported in this paper include eight in tier 1 of the long-continued Jordan fertility experiments of the Pennsylvania Agricultural Experiment Station. The treatment consisted of the following combinations of fertilizers on plots unlimed since the inception of the experiment in 1881: Check, N, P, K, NP, NK, PK, and NPK. The carrier of nitrogen was dried blood; of phosphorus, superphosphate; and of potassium, the chloride, commonly designated as muriate of potash.

Two methods of presenting the results graphically are given, and the indications given by each are discussed.

A relationship exists between the percentage of nitrogen in the dried material of the leaf and in the fertilizer, but none between nitrogen in the leaf and the yield, nor between nitrogen in the fertilizer and the yield except when accompanied by phosphoric acid and potash.

A relationship exists between the percentage of phosphoric acid in the leaf and in the fertilizer; also between phosphoric acid in the leaf and the yield, and between phosphoric acid in the fertilizer and the yield.

No relationship exists between the content of potash in the leaf and in the fertilizer, and none between the variation, however great, of potash in the leaf and the variations in the yield; also there is no relationship between potash in the fertilizer and the yield.

The percentage content of CaO and MgO in the leaves of plants receiving superphosphate without potash (P, NP) was much higher than in those which received potash with the superphosphate (PK, NPK). Moreover the CaO content of the leaves of plants receiving the P and NP treatments differed but little from the CaO content of the leaves from plants which received lime only.

Two concepts peculiar to the method are introduced, viz, that of quantity (intensity of nutrition) and that of quality (physiological ratios of the elements). The latter is conveniently expressed as an NPK unit representing the equilibrium between N–P_2O_5–K_2O in the leaf selected at the moment of sampling. A fertilizer may intervene to affect a change in the (1) quantity or (2) quality or in (1) and (2) simultaneously.

Low intensities of nutrition are associated with low yields (check, P, N, NP) and high intensities with high yields (PK, NPK); but when "luxuskonsumption" of potash occurs, relatively low yields may accompany relatively high intensities of nutrition (K, NK).

The succession of the coordinate points of the NPK unit of the third leaf from the base taken at four sampling periods from plants growing in the differently treated plots are shown in trilinear coordinates.

The treatments fall into three groups according to their position on the triangle—i. e., according to the composition of the respective

NPK units: Group 1, poorly nourished plants (check NK, N, K) characterized by low P_2O_5 in the NPK unit; group 2, better nourished plants (P, NP) with very high P_2O_5, high N, and very low K_2O in the NPK unit; and group 3, best nourished plants (PK, NPK), with values for N, P_2O_5, and K_2O intermediate between those of groups 1 and 2.

LITERATURE CITED

(1) LAGATU, HENRI, and MAUME, LOUIS, with the collaboration of CROS, LUCIENNE, DELOCHE, LUCIE, and FABREGUETTES, MARCELLE.
n. d. LE DIAGNOSTIC FOLIAIRE DE LA POMME DE TERRE. DEUXIÈME MÉMOIRE. Ann. École Nat. Agr. Montpellier (n. s.) 22: [50]–158, illus.

(2) NOLL, C. F., GARDNER, F. D., and IRVIN, C. J.
1931. FIFTIETH ANNIVERSARY OF THE GENERAL FERTILIZER TESTS. A SUMMARY OF RESULTS OBTAINED WITH COMMERCIAL FERTILIZERS, MANURE, LIME, AND LAND PLASTER DURING A PERIOD OF 40 YEARS. Pa. Agr. Expt. Sta. Bull. 264, 24 pp., illus.

(3) THOMAS, WALTER.
1934. MISCONCEPTIONS RELATIVE TO THE MINERAL COMPOSITION OF PLANTS. Science 80: 587.

(4) ———
1936. MATHEMATICAL EXPRESSION OF EQUILIBRIUM BETWEEN NITROGEN AND PHOSPHORIC ACID IN PLANTS. Science 84: 422–423, illus.

(5) ———
1937. FOLIAR DIAGNOSIS: PRINCIPLES AND PRACTICE. Plant Physiol. 12: 577–599, illus.

(6) ———
1938. FOLIAR DIAGNOSIS: ITS RELATION TO THE OPTIMUM NUTRITION OF THE POTATO. Plant Physiol. 13: 677–694.

(7) ——— and MACK, WARREN B: 677–694.
1938. FOLIAR DIAGNOSIS IN RELATION TO DEVELOPMENT AND FERTILIZER TREATMENT OF THE POTATO. Jour. Agr. Research 57: 397–414, illus.

(8) ——— and MACK, WARREN B.
1939. FOLIAR DIAGNOSIS: THE INFLUENCE OF THE SOIL ON THE ACTION OF FERTILIZERS. Plant Physiol. (In press.)

A METHOD OF ESTIMATING THE VOLATILE PRODUCTS LIBERATED FROM STORED FRUIT [1]

By FISK GERHARDT, *physiologist*, and BOYCE D. EZELL, *assistant horticulturist*, *Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

During maturation and senescence, most fruits produce characteristic odorous constituents. Under improper handling and storage practices, certain volatile products of metabolism may form or accumulate in abnormal quantities and become closely associated with definite physiological storage disorders. Common storage scald of apples (*1*) [2] and scald and core break-down of pears (*12*) may have their origin from such causes.

Power and Chesnut (*13*, *14*) have shown that the odorous constituents of apples consist chiefly of the amyl esters of formic, acetic, caproic, and caprylic acids, and a considerable proportion of acetaldehyde and ethyl alcohol. Ethylene (*3*, *7*, *11*), geraniol (*14*), and limonene (*9*) have also been identified as normal volatile products of fruit metabolism. These odorous constituents are all derivatives of three large classifications in structural organic chemistry, namely, the aldehydes, alcohols, and esters of the fatty acid series; the olefines; and the heterogeneous group of essential oils, many of which contain unsaturated hydrocarbons that are usually termed terpenes. These compounds are all chemically reactive and are easily oxidized. They either form addition compounds or undergo polymerization or saponification in the presence of a strong mineral acid. These compounds, therefore, should be capable of absorption in concentrated sulphuric acid and subsequent oxidation with suitable oxidizing reagents.

Many of these volatile components that comprise the odorous constituents of stored fruit are present only in minute amounts. Furthermore, the presence of the various types of individual components may be conditioned by a specific type of physiological metabolism, handling practice, or storage temperature. Acetaldehyde has been identified as one of the more common and more abundant volatile products of fruit metabolism (*12*, *13*). This compound can be readily obtained in pure form and is also subject to accurate chemical estimation. For these reasons acetaldehyde has been chosen as representative of the odorous constituents of stored fruit and has been used as a uniform reference standard in the experimental work herein described.

This paper presents a method for the determination of the total oxidizable volatile constituents emanating from stored fruit. The method is based on the absorption of these materials in concentrated sulphuric acid, oxidation with ceric sulphate, and evaluation in terms of ceric sulphate reduced. Several applications of the method are described. This method of measuring volatiles requires the use of

[1] Received for publication July 22, 1938.
[2] Italic numbers in parentheses refer to Literature Cited, p. 502.

simple and inexpensive laboratory equipment, while the necessary reagents are easily prepared and are stable for long intervals of time. The method lends itself to simultaneous routine analysis of a large number of samples and can also be used in conjunction with respiratory measurements of a common lot of fruit. It possesses the accuracy and ease of end-point titration that are characteristic of all iodometric procedures.

REVIEW OF METHODS FOR THE DETECTION OF VOLATILE PRODUCTS FROM FRUITS

Power and Chesnut (*13*) passed the gases emanating from stored Nero apples through a saturated solution of sodium bisulphite. After neutralization and steam distillation of this solution, the presence of acetaldehyde in the distillate was established by the delicate Rimini (dimethylamine) test. Thomas (*19*), using aqueous acid solutions of phloroglucinol, was able to trap and identify acetaldehyde as one of the volatile components liberated from Fen Newtown apples. Volatile substances from ripe apples have been shown by Elmer (*2*) to inhibit normal sprouting of germinating potatoes. He identified the inhibitory substance as ethylene (*3*). Gane (*7*), by absorption in bromine and by preparation of crystalline N. N. diphenylethylene diamine from ethylene dibromide, was able to establish chemically the presence of ethylene among the gaseous products liberated from ripe apples.

Biological assay by means of epinastic responses with Bonny Best tomato plants led Hansen and Hartman (*11*) to surmise that ethylene may be evolved during the ripening of Anjou pears. These investigators were also able to establish the presence of ethylene by chemical means. Oxidation of the gaseous emanations from pears was accomplished by passage through iodine pentoxide at 150° C. Ethylene, as calculated from the iodine liberated, was present in a concentration of approximately 1 part by volume to 70,000 parts of air. Specific solubility of the gaseous emanation in 20-percent mercuric nitrate (2 N in nitric acid) and differential solubility in 87-percent sulphuric acid established the chemical identity of ethylene.

In 1931 Gane (*6*) attempted to concentrate the odorous constituents from the air in a model fruit-storage chamber. Steam distillation of 14 pounds of "drip" from the roof grids gave a clear distillate, the ether extract of which yielded 0.05 gm. of gummy material. In another experiment air from this chamber was drawn through two receivers cooled in solid carbon dioxide and then through two additional collecting tubes immersed in liquid oxygen. Two one-hundredths of a gram of a waxlike material was obtained from the ether extraction of 29 gm. of water in the two former receivers. Odorous substances escaped from the tubes immersed in liquid oxygen immediately upon removal from the lower temperature. Five-tenths of a cubic centimeter of a clear liquid with a very strong odor of apples remained after these receptacles attained room temperature.

Ozone can be determined by its decomposition of aqueous potassium iodide. Gane (*8*) attempted to use this fact as a basis for a method of measuring the volatile emanation from ripe bananas by passing a stream of air carrying a low concentration of ozone over the fruit and estimating the residual ozone after its partial destruction by reaction with the volatiles. He was unable to detect any loss of ozone, how-

ever, and intimated that other substances with a carbonyl group may have been present in the air stream and could react with the ozone to form an unstable peroxide which in the presence of water would also liberate iodine from potassium iodide.

Teague (*18*) used iodine pentoxide at 150° C. for the estimation of carbon monoxide in motor-exhaust gas; the method was based on the following reaction:

$$I_2O_5+5CO=5CO_2+2I$$

The iodine from this reaction was collected in aqueous potassium iodide and titrated with 0.001 N sodium thiosulphate. Gane (*8*) studied the oxidation of the volatile emanations from stored apples by passing the air stream over iodine pentoxide heated at 150° C. From the iodine equivalent in terms of carbon dioxide formed during the reaction, Gane concluded that the oxidation was incomplete and that iodine pentoxide could not be used satisfactorily for the estimation of volatile substances produced by apples.

Gane (*8, 10*) finally employed a combustion method to measure the volatile emanation from oranges, apples, and bananas. The method was used in conjunction with respiration studies. After absorption of the respiratory carbon dioxide in flaked sodium hydroxide, the air was passed through a tube of copper oxide heated to 800° C. The organic materials in the air stream were oxidized to carbon dioxide and water; the former was then absorbed in a standardized solution of sodium hydroxide. The rate of air movement through the system did not exceed 0.9 liter per hour, so that the time available for combustion was approximately 1 minute.

SELECTION AND STANDARDIZATION OF OXIDIZING SOLUTION AND SUBSTRATE

Since acetaldehyde has been identified as one of the volatile products liberated from fruit (*13*), and since it can be determined volumetrically with ease and accuracy (*16*), it was used as the reference standard for establishing the optimum conditions for the oxidation of typical volatile emanations from fruit. Ceric sulphate was chosen as the oxidizing agent because of its powerful oxidizing action (*4*), versatility (*15, 21*), stability (*20*), and adaptability to the problem.

The oxidizing solution, 0.1 N in ceric sulphate and 0.5 M in sulphuric acid, was prepared from the anhydrous ceric sulphate and standardized against Bureau of Standards arsenic trioxide, iodine monochloride being used as a catalyst according to the method of Swift and Gregory (*17*). Ceric sulphate solutions of this strength are stable for a year, are not sensitive to light or air, and are not changed by boiling for 1 hour (*20*). Acetaldehyde solutions were standardized according to the bisulphite method of Ripper as given by Sutton (*16*).

Furman and Wallace (*5*) have shown that it is possible to determine thiosulphate in solutions or in salts by titration with standard ceric sulphate solution if an excess of potassium iodide is present. Two precautions must be observed, however: (1) The initial volume for titration should be at least 10 times the volume of thiosulphate used, and (2) the solution for titration must be sufficiently acid (0.3 to 0.5 M in H_2SO_4) to insure a permanent starch iodide end point.

With these precautions in mind, it was possible to obtain comparable standardization values for ceric sulphate by titration with either 0.1 N (Bureau of Standards) arsenic trioxide or with 0.1 N sodium thiosulphate.

The reduction-oxidation changes of ceric sulphate in the experiments reported here were determined by titration against 0.1 N sodium thiosulphate in the presence of excess potassium iodide in acid solution according to the method of Furman and Wallace (*5*).

EXPERIMENTAL PROCEDURE

OXIDATION OF ACETALDEHYDE WITH CERIC SULPHATE

In order to establish the optimum conditions for the oxidation of acetaldehyde, a study was first made of the relative importance of time and temperature. The oxidizing solutions were maintained at a constant volume of 150 cc. and at an acid concentration which was 3 molal in sulphuric acid. The data for these experiments are presented in table 1.

TABLE 1.—*Influence of time and temperature on oxidation of acetaldehyde with ceric sulphate*

CH_3CHO taken (milligrams)	Oxidation		$Ce(SO_4)_2$ (0.1030 N) added	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Reduction-oxidation ratio $\frac{Ce(SO_4)_2}{CH_2CHO}$	Mol. equivalents of $Ce(SO_4)_2$ required per mol. CH_3CHO
	Period	Temperature					
	Hours	*° C.*	*Cubic centimeters*	*Cubic centimeters*	*Milligrams*		*Milligrams*
14	1	24	25	5.0	166.1	11.86	1.6
14	24	24	25	9.5	315.6	22.53	3.0
14	.5	98	50	20.0	664.4	47.45	6.3
14	1	98	50	20.5	681.0	48.64	6.5
14	2	98	50	21.5	714.2	51.00	6.8
14	3	98	50	21.5	714.2	51.00	6.8
14	4	98	50	21.5	714.2	51.00	6.8
0	1	98	50	.0			
0	3	98	50	.0			

Oxidation of acetaldehyde occurred at a very low rate at room temperature; less than one-half of the aldehyde had been oxidized after 24 hours at 24° C. Constant titration values, reduction-oxidation ratios, and mol. equivalents of ceric sulphate were obtained, however, after heating at 98° for 2 hours. All subsequent oxidation studies were standardized to a temperature of 98° and for a time interval of 2 hours. Blank titrations showed no reduction of the ceric to the cerous ion under these conditions of oxidation.

In another series of experiments the oxidation of acetaldehyde was studied further by varying the concentration of ceric sulphate and sulphuric acid and the total volume of the oxidizing solution. The temperature and period of oxidation were held at 98° C. and 2 hours, respectively. The results of this study appear in table 2.

The data in table 2 show that the reduction-oxidation ratios (milligrams of $Ce(SO_4)_2$ reduced per milligram of CH_3CHO oxidized) remain constant over a wide range of variation in acid concentration, excess of oxidizing agent, and volume of oxidizing solution. These results are similar to those reported by Willard and Young (*21*) for the oxidation of tartaric acid with ceric sulphate.

TABLE 2.—*Oxidation of acetaldehyde as influenced by the concentration of $Ce(SO_4)_2$, H_2SO_4, and the total volume of the solution*

Acetaldehyde taken (milligrams)	Total volume	Molarity in H_2SO_4	$Ce(SO_4)_2$ (0.103 N) taken	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Reduction-oxidation ratio $\frac{Ce(SO_4)_2}{CH_3CHO}$
	Cubic centimeters		*Cubic centimeters*	*Cubic centimeters*	*Milligrams*	
14	150	0.5	25.0	20.5	681.0	48.6
14	150	1.0	25.0	21.5	714.2	51.0
14	150	3.0	25.0	21.5	714.2	51.0
14	200	3.0	25.0	21.5	714.2	51.0
14	200	3.0	50.0	21.5	714.2	51.0
14	200	3.0	100.0	21.5	714.2	51.0

A series of oxidations was next carried out in which the acetaldehyde content was varied over a wide range of concentration. The volume of the oxidizing solution was held at 200 cc.; the temperature and reaction time were similar to those for the preceding experiments. Data relative to the oxidation of various concentrations of acetaldehyde appear in table 3.

TABLE 3.—*Influence of concentration of acetaldehyde on its oxidation with ceric sulphate*

Acetaldehyde taken (milligrams)	$Ce(SO_4)_2$ (0.103 N) taken	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Reduction-oxidation ratio $\frac{Ce(SO_4)_2}{CH_3CHO}$	Acetaldehyde taken (milligrams)	$Ce(SO_4)_2$ (0.103 N) taken	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Reduction-oxidation ratio $\frac{Ce(SO_4)_2}{CH_3CHO}$
	Cubic centimeters	*Cubic centimeters*	*Milligrams*			*Cubic centimeters*	*Cubic centimeters*	*Milligrams*	
44.8	100.0	69.7	2,315.4	51.6	11.2	50.0	17.2	573.4	51.2
22.4	100.0	34.7	1,152.7	51.4	5.6	50.0	8.6	285.7	51.0

Attention should be called to the small change in the reduction-oxidation ratio; it bears a positive correlation with the increase in concentration of acetaldehyde and is probably conditioned by the accuracy of the normality factor of the latter. When due consideration is given to the normality weight of ceric sulphate and to its reduction equivalent in terms of acetaldehyde, the increase in the reduction-oxidation ratio in table 3 becomes less significant. Furthermore, a variation of 700 percent in acetaldehyde concentration produced a concomitant change of only 1 percent in this ratio. Willard and Young (*21*), using a wide range in concentration of tartaric acid, obtained a constant ratio of acid oxidized to ceric sulphate reduced. It is the opinion of the authors that this is also the case with acetaldehyde. The small changes in the reduction-oxidation ratio in table 3 in all probability are representative of the variations that may be anticipated under routine conditions, and the milligrams of ceric sulphate reduced per milligram of acetaldehyde oxidized can be considered constant over a wide range in acetaldehyde concentration.

Since it has been shown (*21*) that formic acid is not further oxidized by ceric sulphate, one would anticipate that acetaldehyde would be

oxidized only to formic acid. Theoretically this would require 6 mol-equivalents of cerium according to the following equation:

$$(1)\ 6Ce(SO_4)_2+CH_3CHO+3H_2O\rightarrow 2HCOOH+3Ce(SO_4)_3+3H_2SO_4$$

However, carbon dioxide has been identified as one of the end products in the oxidation of acetaldehyde with ceric sulphate. This fact can be shown in the following equation, where the cerium equivalent is 8:

$$(2)\ 8Ce(SO_4)_2+CH_3CHO+3H_2O\rightarrow HCOOH+CO_2+4Ce(SO_4)_3+4H_2SO_4$$

A cerium equivalent of 6 would require a reduction-oxidation ratio of 45.3, whereas an equivalent of 8 would require a ratio of 60.4. By experiment, however, under standardized conditions, data in tables 1, 2, and 3 show a cerium equivalent of 6.8 and a reduction-oxidation ratio of 51. Obviously stoichiometric relations are not involved in this case and the reaction cannot be expressed by equation. According to Smith [3] what actually happens in this case is that the oxidation of the CHO group is slower than the oxidation of the CH_3 group. Reactions of this type have previously been reported by Willard and Young (*21*).

ABSORPTION AND OXIDATION OF ACETALDEHYDE EMANATIONS

The following experiments are concerned with a quantitative study of the absorption of acetaldehyde in concentrated sulphuric acid and its ultimate determination by oxidation with ceric sulphate. Considerable difficulty was experienced in devising a suitable absorption apparatus to overcome the bothersome problem encountered in the frothing of the sulphuric acid under rapid aeration. Small Truog towers, constructed from heavy glass tubing 22 mm. in diameter and 27 cm. in length, when filled with glass beads and stoppered into 250-cc. extraction flasks, minimized the frothing problem and served as efficient scrubbers.

The amount of acetaldehyde given off was determined by standardizing (*16*) the original solution before and after aeration. The flask containing the acetaldehyde solution was connected to the Truog absorption tower containing 25 cc. of sulphuric acid (specific gravity, 1.84), and the latter was connected through a flowmeter to a vacuum line. The apparatus was held at a temperature of 65° F. and aerated with aldehyde-free air at the rate of 10 liters per hour for a period of 4 days. Four such absorption trains, each bearing a different original concentration of acetaldehyde, were aerated simultaneously.

After aeration for 4 days, the absorption towers were dismantled and the glass beads were washed into the extraction flasks with distilled water. Measured amounts of standardized ceric sulphate were then added and the oxidations were carried out as previously described. Data from these experiments are presented in table 4.

Data in table 4 show that concentrated sulphuric acid is an efficient absorbent for acetaldehyde. Volatiles of this type may be quantitatively removed from an air stream of 10 liters per hour, and may be oxidized directly in the original absorption flask with ceric sulphate. The percentage-recovery values, based on an average ceric sulphate

[3] Private communication from G. Frederick Smith, University of Illinois, Urbana, Ill.

reduction equivalent of 51 mg. for each milligram of acetaldehyde oxidized, are sufficiently great to warrant consideration of this method for the quantitative determination of acetaldehyde. The adaptability of a single acid (H_2SO_4) to the preparation of the ceric sulphate solution, to the absorption and oxidation of the volatile, and even to the final iodometric titration, is a factor of more than passing importance to the analyst.

TABLE 4.—*Aeration of acetaldehyde, absorption in sulphuric acid, and oxidation with ceric sulphate*

CH_3CHO removed by aeration (milligrams)	$Ce(SO_4)_2$ (0.103 N) taken	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Recovery of acetaldehyde based on average R-O ratio of 51	Recovery of acetaldehyde based on that removed by aeration
	Cubic centimeters	*Cubic centimeters*	*Milligrams*	*Milligrams*	*Percent*
6.7	50.0	9.9	328.8	6.43	96.0
12.5	50.0	19.0	631.2	12.37	98.9
25.0	100.0	37.2	1,235.7	24.23	96.9
25.7	100.0	38.4	1,275.6	25.01	97.3
Average recovery					97.2

DETAILS OF PROCEDURE FOR THE PROPOSED METHOD

ABSORPTION OF THE VOLATILES

Small Truog absorption towers, as previously described, filled with glass beads and fitted with 250-cc. extraction flasks, served as efficient gas scrubbers; 25 cc. of concentrated sulphuric acid (specific gravity, 1.84) in these towers has a large absorptive capacity for trapping the volatile emanations from stored fruit. Tests with overripe Bartlett pears have shown the above amount of acid to be sufficient to absorb a quantity of volatiles the oxidation of which would require the reduction of 4.56 gm. of ceric sulphate. The movement of the air stream should be constant and rapid enough to prevent accumulation of the volatiles over the stored fruit.

OXIDATION OF THE VOLATILES

At the close of the absorption period, the towers are dismantled and the glass beads are washed into the extraction flask with 75 cc. of distilled water. Ceric sulphate (0.1 N) is then added; usually 50 to 100 cc. is sufficient for the oxidation and for insuring an excess of the reagent. The resulting solution is approximately 3 molal in sulphuric acid. The flasks are then heated over a boiling water bath for 2 hours (oxidation temperature, 98° C.). At the termination of the oxidation period, their liquid contents are transferred through a glass funnel into a 500-cc. volumetric flask. The glass beads in the funnel are washed with an amount of distilled water sufficient to bring the contents of the volumetric flask to volume. The resulting solution is approximately 1 molal in sulphuric acid.

TITRATION AND EXPRESSION OF RESULTS

Aliquots of 100 cc. from the above-mentioned final volumes are diluted with 250 cc. of distilled water, 0.3 to 0.5 gm. of potassium

iodide are added, and the solutions are titrated to a starch iodide end point with 0.1 N sodium thiosulphate. The amount of 0.1 N sodium thiosulphate, by differential titration of blank and sample, is equivalent to the amount of 0.1 N ceric sulphate reduced. This value (in cubic centimeters multiplied by 33.22 milligrams of $Ce(SO_4)_2$ per cubic centimeters of 0.1 N solution) will express the results in terms of milligrams of ceric sulphate reduced during the oxidation of the volatile substances. It should again be emphasized that the applicability of this method to the measurement of the total volatile emanation from stored fruit is dependent entirely upon the capacity of such substances for undergoing oxidation.

APPLICATIONS OF THE METHOD

Preliminary studies, involving measurement of the volatile emanation from apples when enclosed in 5-gallon wide-mouthed jars and held at 32° F. in a large commercial storage room, indicated that the source of air during aspiration was an important factor. Attempts to remove the volatiles from storage air prior to passage over the experimental fruit, met with little success. Concentrated sulphuric acid was efficient for only a short period, owing to the high concentration of the volatiles in the storage air and the low solubility of their polymerization derivatives in sulphuric acid at 32°, which solidified after a short interval of aspiration. Outside air, subsequently conditioned as to temperature and humidity, was, however, successfully employed as an aspiration medium.

The volatile materials appearing in an aspiration stream of air from three different sources were determined according to the method presented in this paper. Continuous aerations were maintained for a period of 6 days at the rate of 10 liters of air per hour. These data appear in table 5.

TABLE 5.—*Volatile material in air from different sources*

Source of air	$Ce(SO_4)_2$ (0.1 N) reduced	$Ce(SO_4)_2$ reduced	Average concentration of volatiles per cubic foot of air (in terms of $Ce(SO_4)_2$ reduced)
	Cubic centimeters	*Milligrams*	*Milligrams*
Outside air	0.5	16.61	0.32
Outside air conducted by means of 30 feet of light-walled rubber tubing through a commercial apple-storage chamber filled with fruit held at 32° F	5.5	182.71	3.59
Inside storage air from same commercial apple-storage chamber	20.5	681.01	13.40

These analyses are in reality blank or check determinations of air under three different conditions. They serve to emphasize the fact that the composition of the aspiration stream may be of major importance, especially in certain studies of the absorption of volatiles from fruit at low temperatures wherein their rate of emanation is greatly restricted.

The data in table 5 are important from three other standpoints: (1) They show the sensitivity of the proposed method; (2) they indicate that rubber is permeable to the odorous constituents given off by stored fruit (rubber is also known to be permeable to ethylene gas);

and (3) they emphasize the degree of accumulation of these materials that may be anticipated within a well-managed commercial apple-storage room.

Respiration measurements have been made during much of the authors' physiological work dealing with the development of soft scald in apples. These studies, up to the present time, indicate no close correlation between the respiratory intensity or its climacteric and this type of storage disorder. Preliminary experiments have shown that it is possible to measure both the carbon dioxide from respiration and the volatile emanation from apples by passing the carbon-dioxide free air of the aspiration stream first through absorption towers containing concentrated sulphuric acid and then through similar ones containing barium hydroxide. By the adoption of this procedure it should be possible to follow the course of the normal ripening of fruits in terms both of respiration and of emanation of volatile materials.

Golden Delicious apples, being both aromatic and susceptible to soft scald, were harvested at their optimum maturity on October 12, 1937, and held at 65° F. Samples of 100 fruits each were removed to 32° at 5-day intervals, and on December 28, 1937, they were examined for soft scald. Determinations of the respiratory activity and the volatile emanations were made on comparable fruit during storage at 65°. These data are shown in figure 1.

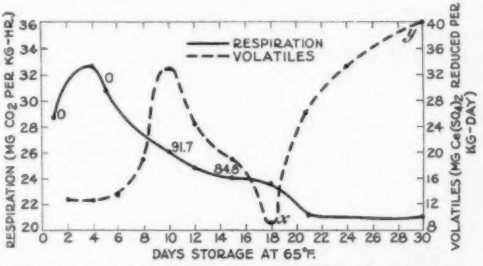

FIGURE 1.—Relation between respiration and emanation of volatiles during ripening of Golden Delicious apples. Numerals on respiration curve indicate percentages of soft scald found December 28, 1937, on fruit that had been removed from 65° F. to 32° after designated number of days. The *x*–*y* portion of volatile curve represents emanation of volatiles after fruit had become infected with penicillium and botrytis rots.

The rate of respiration of Golden Delicious apples (fig. 1) increased rapidly and reached a climacteric on the fourth day. During this interval, however, there was no apparent change in the rate of liberation of volatile material. Respiration continued to decrease with prolonged ripening and in this respect was typical of fruit in the post-climacteric stage of ripening.

The volatile materials began to increase rapidly after 6 days of storage at 65° F. and reached a climacteric at 10 days. In Golden Delicious apples the respiratory climacteric preceded the period of maximum emanation of volatiles by approximately 1 week. The time of occurrence of these two climacterics assumes an added interest when studied in relation to the subsequent development of soft scald in cold storage. This storage disorder did not occur when Golden Delicious apples were transferred from 65° to 32° during the respiratory climax; maximum amounts of soft scald, however, were subsequently observed in lots transferred from 65° to 32° during the period of their greatest volatile emanation. These relations are receiving further study.

Attention should be called to the x–y portion of the volatile–emanation curve in figure 1. The presence of penicillium and botrytis infection in the experimental fruit was first observed on the eighteenth day of storage. As a precautionary measure to insure the elimination of all fungus spores, the aspiration stream at this time was diverted through a 10-inch glass tube of absorbent cotton prior to absorption in sulphuric acid. The progressive development of these initial infections was strikingly reflected in the subsequent rise in the rate of liberation of the volatile substances. Gane (*9*) has stated that this rapid rise in the emanation of volatile substances with the onset of visual decay is due to injury to the fruit rather than to the byproducts of the micro-organisms. Tissue injury, however, usually produces a temporary stimulation of the respiratory processes, which did not occur in this instance, as shown in figure 1. Whatever may prove to be the cause of the rapid liberation of these volatiles with the onset of fungus attack, it would appear that the proposed method for the determination of these materials may also find application in studies of various factors associated with the growth of certain fungi.

SUMMARY

Acetaldehyde has been oxidized by standard ceric sulphate in sulphuric acid solution, and the excess ceric sulphate has been titrated iodometrically with standard sodium thiosulphate. Optimum conditions for the oxidation have been established. The cerium equivalent of acetaldehyde under these conditions is 6.8, and the reaction is empirical rather than stoichiometric.

Acetaldehyde emanations can be trapped from an air stream of 10 liters per hour by sulphuric acid and subsequently oxidized with ceric sulphate. The average recovery of acetaldehyde under these conditions was 97.2 percent.

Details of procedure are given for a method of measuring the emanation of volatiles from stored fruit. The method is dependent upon the absorption of these materials in concentrated sulphuric acid, oxidation with an excess of standard ceric sulphate, iodometric titration of this excess with sodium thiosulphate, and expression of the results in terms of ceric sulphate reduced.

Certain applications of the method have been presented. They include measurements of the volatile substances in air from various sources, interrelation of respiration and liberation of volatile substances from Golden Delicious apples, and the emanation of volatile materials as influenced by the presence of certain fruit-rot fungi.

LITERATURE CITED

(1) Brooks, Charles, Cooley, J. S., and Fisher, D. F.
1919. NATURE AND CONTROL OF APPLE-SCALD. Jour. Agr. Research 18: 211–240, illus.

(2) Elmer, O. H.
1932. GROWTH INHIBITION OF POTATO SPROUTS BY THE VOLATILE PRODUCTS OF APPLES. Science 75: 193.

(3) ———
1936. GROWTH INHIBITION IN THE POTATO CAUSED BY A GAS EMANATING FROM APPLES. Jour. Agr. Research 52: 609–626, illus.

(4) Furman, N. Howell, and Evans, Oliver M.
1929. APPLICATIONS OF CERIC SULFATE IN VOLUMETRIC ANALYSIS. V. POTENTIOMETRIC STUDY OF THE REACTION BETWEEN FERROCYANIDE AND CERIC IONS. Jour. Amer. Chem. Soc. 51: 1128–1133, illus.

(5) FURMAN, N. HOWELL, and WALLACE, JOHN H., JR.
1931. APPLICATIONS OF CERIC SULFATE IN VOLUMETRIC ANALYSIS. IX. THE STANDARDIZATION OF THIOSULFATE SOLUTIONS. DETERMINATION OF THIOSULFATE. Jour. Amer. Chem. Soc. 53: 1283–1288.

(6) GANE, R.
1932. VOLATILE PRODUCTS OF THE METABOLISM OF APPLES. [Gt. Brit.] Dept. Sci. and Indus. Research, Food Invest. Bd. Rept. 1931: 241–242.

(7) ———
1935. IDENTIFICATION OF ETHYLENE AMONG THE VOLATILE PRODUCTS OF RIPE APPLES. [Gt. Brit.] Dept. Sci. and Indus. Research, Food Invest. Bd. Rept. 1934: 122–123, illus.

(8) ———
1935. COMBUSTIBLE GASEOUS PRODUCTS OF FRUIT. [Gt. Brit.] Dept. Sci. and Indus. Research, Food Invest. Bd. Rept. 1934: 124–128, illus.

(9) ———
1936. VOLATILE PRODUCTS OF FRUITS. [Gt. Brit.] Dept. Sci. and Indus. Research, Food Invest. Bd. Rept. 1935: 127–129, illus.

(10) ———
1936. A STUDY OF THE RESPIRATION OF BANANAS. New Phytol. 35: 383–402, illus.

(11) HANSEN, ELMER, and HARTMAN, HENRY.
1935. THE OCCURRENCE IN PEARS OF METABOLIC GASES OTHER THAN CARBON DIOXIDE. Oreg. Agr. Expt. Sta. Bull. 342, 10 pp., illus.

(12) HARLEY, C. P., and FISHER, D. F.
1927. THE OCCURRENCE OF ACETALDEHYDE IN BARTLETT PEARS AND ITS RELATION TO PEAR SCALD AND BREAKDOWN. Jour. Agr. Research 35: 983–993.

(13) POWER, FREDERICK B., and CHESNUT, VICTOR K.
1920. THE ODOROUS CONSTITUENTS OF APPLES. EMANATIONS OF ACETALDEHYDE FROM THE RIPE FRUIT. Jour. Amer. Chem. Soc. 42: 1509–1526.

(14) ——— and CHESNUT, VICTOR K.
1922. THE ODOROUS CONSTITUENTS OF APPLES. II. EVIDENCE OF THE PRESENCE OF GERANIOL. Jour. Amer. Chem. Soc. 44: 2938–2942.

(15) SMITH, G. FREDERICK.
1935. CERIC SULFATE; THE USE OF STANDARD CERIC SULFATE AS MODERN ANALYTICAL CHEMISTRY'S MOST RECENTLY DEVELOPED WIDELY APPLICABLE OXIDIZING AGENT . . . Ed. 3, v. 1, 64 pp., illus. Columbus, Ohio.

(16) SUTTON, FRANCIS.
1924. A SYSTEMATIC HANDBOOK OF VOLUMETRIC ANALYSIS . . . Ed. 11, rev. throughout . . ., 629 pp., illus. Philadelphia, Pa.

(17) SWIFT, ERNEST H., and GREGORY, CARTER H.
1930. THE USE OF IODINE MONOCHLORIDE END-POINT IN VOLUMETRIC ANALYSIS. II. THE TITRATION OF ARSENIOUS ACID WITH PERMANGANATE AND WITH CERIC SULFATE. Jour. Amer. Chem. Soc. 52: 901–906.

(18) TEAGUE, M. C.
1920. THE DETERMINATION OF CARBON MONOXIDE IN AIR CONTAMINATED WITH MOTOR EXHAUST GAS. Jour. Indus. and Engin. Chem. 12: 964–968, illus.

(19) THOMAS, MEIRION.
1925. THE CONTROLLING INFLUENCE OF CARBON DIOXIDE. V. A QUANTITATIVE STUDY OF THE PRODUCTION OF ETHYL ALCOHOL AND ACETALDEHYDE BY CELLS OF THE HIGHER PLANTS IN RELATION TO CONCENTRATION OF OXYGEN AND CARBON DIOXIDE. Biochem. Jour. 19: [927]–947, illus.

(20) WILLARD, H. H., and YOUNG, PHILENA.
1929. CERIC SULFATE AS A VOLUMETRIC OXIDIZING AGENT. IX. PREPARATION AND STABILITY OF SOLUTIONS. Jour. Amer. Chem. Soc. 51: 149–152.

(21) ——— and YOUNG, PHILENA.
1930. CERIC SULFATE AS A VOLUMETRIC OXIDIZING AGENT. XI. THE OXIDATION OF ORGANIC ACIDS. Jour. Amer. Chem. Soc. 52: 132–142.

SOIL INFESTATION BY OPHIOBOLUS GRAMINIS AND ITS SPREAD [1]

By HURLEY FELLOWS, *associate pathologist*, and C. H. FICKE, *junior pathologist*, *Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The infestation of soil by a soil-borne parasite and the increase and spread of the parasite following introduction constitute a very complex process.

The object of the experiments described in this paper was to determine whether *Ophiobolus graminis* Sacc. may be spread by some of the means commonly known to be effective for similar plant pathogens. These include spread by living plants, by the transportation of infested soil or diseased host particles, by implements, and by the natural action of wind and water.

METHODS

EXPERIMENTAL INFESTATION OF SOIL

The methods used in the experimental infestation of noninfested soils comprised contamination with naturally infested field soil; transfer of infestation by the diseased host, living and dead; and infestation by means of cultures on organic media.

Infested and noninfested soils were brought in contact in several ways in an attempt to simulate possible field infestation such as is produced by soil blowing and by water-borne soil particles. Various measured mixtures of the two soils were also used. Another opportunity for infestation was provided by placing the two soils in contact without mixing.

Tests for the transfer of infestation by the living host plant were made by arranging the experiments in such a way that the organism might progress along living roots from infested to noninfested soil.

The presence of infestation in any soil was indicated by lesions on Kanred wheat grown therein.

Accidental contamination of noninfested soils in the greenhouse was avoided by formaldehyde sterilization of benches and sand beds. Control pots were kept at some distance from infested soil to prevent contamination by water splashing. An experiment was conducted to determine whether accidental contamination was occurring in the greenhouse during the progress of the investigations. Each year the same pots containing noninfested soil were placed among those containing infested soil and it was found that no accidental contamination occurred.

[1] Received for publication August 10, 1938. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Kansas Agricultural Experiment Station. Contribution No. 357, Department of Botany and Plant Pathology, Kansas Agricultural Experiment Station.

OBSERVATION AND RATING OF DISEASE SEVERITY

All the disease data on plants infected with take-all are expressed in terms of percentage when possible. These percentage readings were evaluated according to the estimated importance of the particular thing observed in relation to plant injury. For example, death, the maximum injury to a plant, was given a value of 5.0; while the percentage of roots diseased, being much less important, was given a value of 1.0. The various items, with their respective values, are given in the following tabulation. The severity of the lesions on the crown, culms, and roots was rated on a scale of 0 to 100 percent according to the judgment of the observer; the extent of the lesions and the density of their color were the chief determining factors.

Item:	*Value*	Item:	*Value*
Percentage of plants tillering	0.1	Average severity of the disease on crowns	2.5
Percentage of culms heading	.1	Average severity of the disease on culms	.1
Percentage of plants dead	5.0	Average severity of the disease on roots	1.0
Percentage of crowns diseased	2.0		
Percentage of culms diseased	3.0		
Percentage of roots diseased	1.0		
Percentage of roots retained	.2		

The percentage readings were multiplied by the evaluation numbers. The sum of the products that may become greater as the disease becomes more severe (e. g., crowns diseased) was divided by the sum of the products that may become less the more severe the disease becomes (e. g., root retention and tillering). The quotient is the final rating for disease severity. Some observations, such as height of plant, could not be treated in such a manner; therefore the final rating was altered according to the judgment of the worker.

Different environmental conditions may affect such factors as tillering or percentage of culms producing heads, irrespective of the amount of disease present. This source of variation must be taken into account and judged on the basis of the behavior of the controls. In some cases, the omission of certain values is necessary. For example, a few plants in a group may be so badly injured that no culms are formed. In such cases, culm evaluations cannot be used in calculating ratings.

The percentage of culms heading did not appear to be materially affected unless the disease was very severe. Accordingly, this was omitted from the calculations unless the plants were badly injured. Comparison of ratings of similar experiments from year to year was possible only in a general way, as it was not always feasible to make the readings at the same stage of maturity. Furthermore, soils taken from different places in the field and at different times differed in severity of infestation.

A rating of 10 is approximately the dividing line between slight and severe injury.

TRANSFER OF INFESTATION IN SOIL

Contact between infested and noninfested soil is necessary before infestation can be transmitted, and the extent of infestation may be modified by the degree of contact. This can be regulated by mixing various proportions of infested soil with noninfested soil or by arranging the type of contact without mixing. The spread of the infestation through the roots of the host also may be a possibility.

TRANSFER BY MIXING SOILS

When the means by which infested soil may ordinarily be carried to a noninfested portion of a field are considered, it is clear that the resulting mixture will be very dilute. It is thus important to know whether lightly contaminated soil will produce diseased plants, and, if so, to know (1) whether infestation becomes greater with successive cropping, and (2) the readiness and means of fungus spread through the soil.

Tests for the transfer of *Ophiobolus graminis* from one soil to another by mixing were made in two ways, each of which consisted in mixing a given quantity of noninfested soil with infested soil. In the first method, different quantities of infested and noninfested soil were mixed to determine the effect of the proportion of infested soil on subsequent infection. This work was done in the greenhouse. In the second method, the quantity of infested soil in the mixture was very small, being such as might be carried by the natural action of wind and surface water. This work was done both in the field and in the greenhouse.

The ability of lightly contaminated soil to produce badly diseased plants was investigated by mixing infested field soil in various proportions with noninfested soil, usually sterilized, and growing Kanred wheat (*Triticum aestivum* L.; syn. *T. vulgare* Vill.) in it. In one case, the infested soil was mixed with unsterilized noninfested soil for a comparison with sterilized soil. All tests were conducted in the greenhouse.

Infested soil was mixed with noninfested soil in proportions of 75, 66, 50, 33, 25, and 15 percent, by volume. Infested and noninfested soil was used separately for controls. In each test the soil was placed in 8-inch clay pots, and 12 seeds of Kanred wheat, a susceptible, hard red winter variety, were planted in each pot. When the plants in noninfested soil had headed, they were removed and the data on the severity of the disease were recorded. This experiment was continued for 3 years, new lots of infested and noninfested soil being used each year. The data are given in table 1.

TABLE 1.—*Severity of take-all on Kanred wheat plants grown in the greenhouse in noninfested sterilized soil and in various percentages of naturally infested soil mixed with noninfested sterilized soil, Manhattan, Kans., 1931–34*

Type of soil	Tests	Plants	Average height of plants	Plants tillering	Culms		Plants dead	Diseased—			Roots retained	Disease severity			
												Average on—			
					Total	Heading		Crowns	Culms	Roots		Crowns	Culms	Roots	Final rating
	No.	*No.*	*In.*	*Pct.*	*No.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
Noninfested soil	27	324	32.0	59.7	722	56.6	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
Infested soil (percent):															
100	19	228	17.2	35.4	329	15.0	30.1	94.4	63.5	92.4	32.1	90.0	73.6	84.9	82.4
75	8	91	22.4	22.0	124	22.6	23.9	70.5	56.6	66.6	51.6	71.3	35.3	66.5	50.6
66	8	98	22.9	32.2	130	21.7	20.3	97.7	61.1	92.8	43.6	77.8	50.0	76.2	60.6
50	8	95	25.8	37.8	159	29.6	9.1	73.2	50.8	67.1	64.6	63.9	43.2	64.4	31.2
33	8	94	30.5	26.6	128	66.3	1.0	50.8	22.4	40.3	80.0	41.4	28.8	32.3	14.0
25	8	92	31.0	54.1	195	58.3	1.2	25.6	11.1	18.4	90.4	35.1	20.8	56.1	8.3

The results from pots containing only 15 percent of infested soil are not included in table 1, since only a trace of disease appeared on the roots and there was no appreciable damage to the plants. The results were very similar whether the noninfested soil was sterilized or not. The plants in these experiments were only slightly injured when the proportion of infested soil was less than 33 percent. As moisture conditions in the field are less favorable for the disease than those ordinarily occurring in the greenhouse, undoubtedly a greater proportion of infested soil would be necessary in the field than in the greenhouse for severe damage to take place the first year.

An experiment was conducted in the greenhouse to determine whether the disease severity would increase with repeated cropping of a lightly infested soil. Sterilized soil was mixed with 15 and 30 percent of infested soil. Kanred wheat was grown in the 15-percent mixture in each of 4 years and in the 30-percent mixture in 5 years. The results are given in table 2. In the 30-percent mixture the severity of the disease increased in general but fluctuated considerably from year to year. The wheat was severely injured in the fifth year, but not so much as in the 100-percent infested control soil. There was slight damage in the 15-percent infested soil, but in no case did the final severity rating exceed 3.1.

Experiments to test the possibility of the transfer of the fungus in soil carried by surface water or winds were conducted in the greenhouse and in the field. Water suspensions from infested soil, or dry infested soil, were used as inoculum. Both sterilized and unsterilized noninfested soil was used, the former being sterilized with steam for the greenhouse experiment and with formaldehyde for the field experiment. The water suspensions were prepared by stirring the infested soil vigorously for one-half hour with an abundance of water and pouring off the supernatant, turbid suspension. Two suspensions were used, one being very turbid and the other being allowed to settle for 1 hour. In the greenhouse tests, one application of each of the two suspensions was applied separately to the noninfested soil nearly to the point of saturation and then the soil was mixed and placed in pots in which Kanred wheat was grown.

Two series of these greenhouse experiments were conducted during different years, in which water suspensions of infested soils from different locations were added to unsterilized noninfested soil. In one experiment (series 1a) no disease was produced, while in the other (series 1b) severe disease occurred only where the very turbid suspension had been applied. The detailed data on this experiment are not presented.

In another greenhouse experiment (series 2, table 3) separate soil lots were similarly inoculated with slightly turbid and with very turbid suspensions of infested soil, and two crops of wheat were grown in each of them. In the first crop no disease developed, but in the second it was abundant. The data from the second crop are given in the first part of table 3.

It is evident from these data that under greenhouse conditions infestation carried by water suspensions of infested soil may become well established in 2 crop years. Contrary to the results of the earlier experiment, the noninfested soils to which the slightly turbid suspensions were added became more heavily infested than those inoculated with the very turbid suspensions.

TABLE 2.—*Severity of take-all in five crops of Kanred wheat grown successively in the greenhouse in the same samples of (1) noninfested sterilized soil or (2) noninfested sterilized soil containing different percentages of naturally infested soil, Manhattan, Kans., 1931–36*

Type of soil and crop year	Tests	Plants	Average height of plants	Plants tillering	Culms			Plants dead	Plants having diseased—		Diseased roots	Roots retained	Disease severity			
					Total	Heading	Average per tillering plant		Crowns	Culms			Average on—			Final rating
													Crowns	Culms	Roots	
Noninfested soil:	*Number*	*Number*	*Inches*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
1931–32	6	86	30.6	82.5	249	53.8	3.0	0	0	0	0	100.0	0	0	0	0
1932–33	6	54	30.8	98.1	218	72.0	4.1	0	0	0	0	100.0	0	0	0	0
1933–34	6	49	25.3	53.0	88	79.5	2.5	0	0	0	0	100.0	0	0	0	0
1934–35	6	61	27.8	73.7	146	40.4	2.9	0	0	0	0	100.0	0	0	0	0
1935–36	3	25	27.3	76.0	77	31.3	3.7	0	0	0	0	100.0	0	0	0	0
Total or average	27	275	28.3	76.6	778	55.4	3.2	0	0	0	0	100.0	0	0	0	.0
15 percent of infested soil:																
1932–33	1	10	32.0	100.0	56	53.5	5.6	0	0	0	T [1]	100.0	0	0	5.0	T
1933–34	1	26	23.0	69.2	47	80.8	2.2	0	5.0	0	3.3	98.0	25.0	0	9.0	1.2
1934–35	1	31	23.0	48.3	52	57.6	2.4	0	66.6	0	T	-------	5.0	0	5.0	3.1
1935–36	1	14	23.0	71.4	25	24.0	2.1	0	T	0	T	100.0	T	0	T	T
Total or average	4	81	25.2	72.2	180	53.9	3.0	0	17.9	0	.8	99.3	7.5	0	4.7	[2] 1.8
30 percent of infested soil:																
1931–32	6	29	27.5	41.1	55	40.8	3.2	3.4	59.5	41.6	51.5	64.0	74.3	62.8	65.3	13.8
1932–33	6	45	18.3	82.2	118	18.6	3.0	6.6	100.0	83.0	100.0	31.4	86.1	39.0	87.7	10.8
1933–34	6	36	25.5	0	44	0	-------	0	73.0	0	25.0	95.0	23.0	0	16.3	24.4
1934–35	5	55	27.6	34.5	73	64.3	1.9	12.7	95.3	2.0	25.4	-------	37.5	73.5	56.2	14.6
1935–36	4	35	14.5	11.4	40	(3)	2.3	17.1	100.0	0	78.7	62.5	55.0	0	42.8	49.4
Total or average	27	200	22.6	33.8	330	30.9	2.6	7.9	85.5	25.3	56.1	63.2	55.1	35.0	53.6	[2] 28.4
100 percent of infested soil:																
1931–32	9	70	16.2	31.4	118	16.1	3.2	44.2	100.0	82.4	98.9	23.5	90.0	67.1	83.2	34.9
1932–33	4	33	19.5	21.2	40	12.5	2.0	6.0	100.0	52.4	99.6	45.0	84.0	35.5	78.0	37.0
1933–34	5	56	20.6	5.3	59	89.9	2.0	14.2	89.5	48.8	90.1	55.9	51.0	27.1	61.6	135.6
1934–35	3	33	19.0	15.1	40	12.5	2.4	24.2	100.0	62.6	99.1	-------	79.5	50.5	88.8	60.2
1935–36	6	47	15.0	0	47	17.0	0	14.8	100.0	40.0	100.0	55.8	78.3	35.2	60.4	75.3
Total or average	27	239	18.0	14.6	304	29.6	1.9	20.6	97.9	57.2	97.5	45.1	76.5	43.0	74.4	[2] 61.4

[1] T=trace.

[2] Derived from averages according to tabulation on p. 506.

[3] Too immature for heads.

138256—39——3

TABLE 3.—*Severity of take-all on Kanred wheat grown in the greenhouse in nonsterilized or sterilized soil previously inoculated in the field or in the greenhouse, Manhattan, Kans., 1935–36*

Series No.	Soil and soil inoculation	Tests	Plants	Average height of plants	Plants tillering	Culms			Plants dead	Diseased—			Roots retained	Disease severity			
						Total	Heading	Average per tillering plant		Crowns	Culms	Roots		Average on—			Final rating
														Crowns	Culms	Roots	
		Number	*Number*	*Inches*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pct.*	*Pct.*
2	Noninfested soil sterilized and inoculated in the greenhouse with suspension—18 months previous to observations:																
	Slightly turbid	2	27	19.0	62.9	46	21.7	2.1	7.3	66.6	52.5	65.8	55.2	90.2	66.0	90.2	37.0
	Very turbid	2	17	18.5	10.0	59	38.9	3.5	.0	50.0	.0	49.0	84.0	84.3	.0	38.7	11.8
2	Noninfested soil sterilized and inoculated in the greenhouse with suspension—30 months previous to observations:																
	Slightly turbid	3	24	18.3	95.8	102	50.0	4.4	.0	100.0	62.5	93.3	57.0	73.9	59.3	61.4	46.9
	Very turbid	3	25	18.3	96.0	121	21.4	5.0	.0	80.9	51.8	67.8	61.4	62.5	42.1	57.8	25.2
2	Noninfested soil not sterilized but inoculated in the greenhouse with suspension—30 months previous to observations:																
	Slightly turbid	4	44	15.7	77.2	143	.6	3.9	13.6	100.0	72.9	96.8	53.7	70.3	53.6	64.6	45.0
	Very turbid	2	21	13.0	76.1	71	.0	4.1	.0	100.0	53.8	99.0	66.8	69.2	42.7	49.2	32.8
3	Noninfested soil sterilized and inoculated in the field in 1931 with—																
	Suspension of infested soil	4	45	29.2	53.3	80	48.5	2.5	.0	100.0	.0	16.2	97.5	33.1	.0	15.0	10.6
	Dry infested soil	4	40	30.7	55.0	80	51.2	2.8	.0	91.6	.0	15.0	96.5	34.0	.0	25.0	10.3
3	Noninfested soil not sterilized but inoculated in the field in 1931 with—																
	Suspension of infested soil	4	39	28.2	53.8	77	51.9	2.8	.0	100.0	.0	18.7	94.7	28.7	.0	38.7	11.2
	Dry infested soil	4	40	33.0	47.5	65	61.5	2.3	.0	97.7	.0	15.0	97.2	44.0	.0	31.2	11.6
	Controls grown in—																
	Soil sterilized before seeding in 1935	6	61	27.8	73.7	146	30.8	2.9	.0	.0	.0	.0	100.0	.0	.0	.0	.0
	Naturally infested soil	6	61	12.1	8.1	66	.0	2.0	27.8	100.0	75.8	98.8	42.4	77.0	47.2	75.4	103.1

In the field experiments, an area free of infested soil was chosen and four similar plots, each 3 feet square, were spaded and separately enclosed by boards 1 by 8 inches with their edges sunk into the soil about 3 inches. Two of these plots were sterilized with formaldehyde, and the other two were left unsterilized. One of the sterilized and one of the unsterilized plots were inoculated with one application of the very turbid suspension from infested soil applied to the surface with a sprinkling can. The remaining two plots were inoculated with one application of dry, well-pulverized infested soil taken from known infested spots. This pulverized soil was scattered lightly over the surface of the plots and raked into the top ½-inch layer.

The four plots were then cropped with wheat successively for 3 years. During this period no trace of take-all appeared at any time in any of them. Unfortunately, it then became necessary to discontinue the field observations, but the soil was removed to the greenhouse and placed in pots and the cropping with wheat was continued for 2 years. The first crop in the greenhouse (1934–35), the fourth year after inoculation, showed no sign of disease, but the second crop was diseased. The data from this crop (1935–36), together with those for the controls grown in sterilized and naturally infested soil, are given in the last part (series 3) of table 3. The damage was not very great but was sufficient to show that water-borne and wind-blown soil may carry the fungus under field conditions.

TRANSFER BY CONTACT OF SOILS WITHOUT MIXING

Since infested areas in a field often increase in size, it seemed desirable to determine how the fungus finds its way from infested to adjoining noninfested soil and the rate at which this movement takes place. An attempt to study this was made by placing infested and noninfested soils in contact without mixing them. Experiments were conducted both in the field and in the greenhouse.

For the field experiments, two fields known to be free from take-all were selected and four holes 1 foot square and 1 foot deep were dug in each. These holes were filled with infested soil and wheat was planted in this and the surrounding soil. In another noninfested field a hole 12 feet square and 1 foot deep was similarly filled with infested soil, which was cropped to Kanred wheat for 5 successive years.

At the end of the first season, the wheat growing in the 1-foot cubes of infested soil was examined and found to be free of lesions, as was also the wheat in the surrounding soil. In the 12-foot-square plot of infested soil, take-all occurred on the plants the first year in a spot about 4 feet in diameter in the center of the area. The second year a few diseased plants appeared in the center of the same spot. In the 3 following years, all plants were free of take-all.

In the greenhouse experiments, infested soil was arranged in separate boxes so that it was over, under, and against one side of noninfested soil. No wheat was planted, but the soil was kept constantly moist. In one experiment the soils were kept in contact for 2 years and in another for 1 year. In the 2-year experiment the infested soil was renewed at the end of the first year. At the end of the respective periods, the originally noninfested soils were removed in layers or sections taken at increasingly greater distances from the place of contact with infested soil and were tested for the presence of *Ophiobolus graminis* by growing wheat in them. Wheat plants in the noninfested

soil never became infected regardless of the position of the noninfested soil with respect to the infested soil or the period of contact.

Wheat was also grown in the infested soil after contact to test its potency for disease production. At the end of the 1-year experiment of eight infested soil samples, four had lost their infestation, one had lost most of it, and three had retained it. In the 2-year experiment the infested soil that had been renewed at the end of the first year retained the infestation.

TRANSFER BY HOST PLANT

It was shown previously [2] that *Ophiobolus graminis* may advance along a wheat root. Accordingly, experiments were planned to find whether the roots may be a means of spreading infestation.

All of the experiments were conducted in the greenhouse. Well-mixed infested soil was placed in boxes (12 by 18 by 4 inches for series 1 to 3 and 12 by 12 by 12 inches for series 4 and 5) in such a way that it was against one side of noninfested soil or over or under it. These arrangements were maintained for 2 successive crop years, but the infested soil was replaced with fresh infested soil between crops. This was done to insure as nearly as possible an ever-present supply of infested soil, since, in greenhouse experiments, as shown above, infested soil in contact with noninfested soil soon lost its infestation.

In series 1, 2, and 3, the 12- by 18- by 4-inch boxes were filled two-thirds of their length with noninfested soil and the remaining one-third with infested soil, care being taken to avoid mechanical mixing at the plane of contact when the boxes were filled. For series 4, a 5-inch layer of noninfested soil was placed in the bottom of the 12- by 12- by 12-inch boxes and then a 5-inch layer of infested soil on top of it. In series 5, these layers were placed in the reverse order.

Where infested and noninfested soil were placed in contact side by side, one series (series 1) was left uncropped for 2 years; in a second series (series 2), the infested soil only (series 1a) was cropped for 2 years; and in a third series (series 3), the noninfested soil only (series 3b) was cropped for 2 years. In series 2 and 3 the wheat roots spread to both kinds of soil. In the vertical experiment wheat was planted in one case in infested soil (series 4a) and in the other in noninfested soil (series 5b), with the roots penetrating both soils. The arrangement of the soils and cropping is shown in figure 1. At the end of the period, the soil that was originally noninfested was removed in sections taken at increasingly greater distances from the plane of contact with the infested soil. Each section was potted separately and planted to wheat. Samples of the infested soil used in each arrangement were also potted and cropped with wheat, and the crop was examined in the usual manner for symptoms of take-all.

The results of the experiments in which the infested and noninfested soils were arranged side by side are given in table 4 and those in which they were arranged in layers in table 5. It will be noted in table 4 that, irrespective of the distance from the infested soil, there was no lateral spread of the fungus from infested to noninfested soil unless wheat plants were grown in one or the other with the roots penetrating both soils. This is in agreement with a similar experiment mentioned above. On the other hand, the evidence of spread in the cropped

[2] FELLOWS, HURLEY, and FICKE, C. H. EFFECTS ON WHEAT PLANTS OF OPHIOBOLUS GRAMINIS AT DIFFERENT LEVELS IN THE SOIL. Jour. Agr. Research 49: 871-880, illus. 1934.

soil to a distance of 12 inches is unmistakable. It is of special interest to note that the fungus spread more when the wheat roots grew from noninfested into infested soil than when they grew from infested into noninfested soil. This was probably due to the greater volume of roots formed by the plants in the noninfested soil, as they were healthy in their early stages of growth while the others were not.

The vertical spread from infested to noninfested soil was also much greater when the wheat roots grew from the originally noninfested soil into the infested soil (table 5). In fact, there was practically no vertical spread from infested to noninfested soil when the wheat roots grew from the infested soil into the noninfested soil (series 4b).

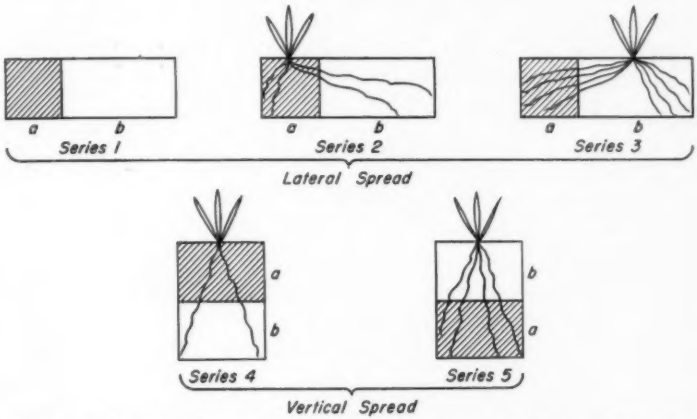

FIGURE 1.—Diagram of methods used in testing the spread of *Ophiobolus graminis* from infested to noninfested soil in infected roots of living wheat plants: *a*, Infested soil; *b*, noninfested soil. (Data in tables 4 and 5.)

The infested soil was not so heavily infested after having been in contact with noninfested soil as it was originally. For example, in the experiment relating to lateral spread (table 4) the original infested soil grew plants with a disease rating of 110.0, whereas after contact with the noninfested soil the disease ratings were 32.6, 44.8, and 67.7 (table 4); in the experiment relating to vertical spread the severity rating after contact was 93.4 and 82.9. These results are in accord with the results of field experiments previously mentioned.

As the plants were grown in soil taken at progressively greater distances from the infested soil against which it rested, a gradual decrease of disease would be expected. In general, this was found to be true but there were some irregularities. These may be due to experimental error or perhaps to the same factors, whatever they are, that sometimes cause infested soils to lose their infestation after having been in contact with noninfested soils.

It is possible that dead material from diseased host plants may harbor the parasite. Such material may be readily carried by farm implements. Large deposits of straw and stubble may accumulate in spots as a result of the periodic cleaning of harrows. The possibility that diseased host remains may carry infestation was tested in

TABLE 4.—*Lateral spread of Ophiobolus graminis in soil as judged by the severity of take-all on Kanred wheat grown in the greenhouse in samples of (1) infested soil and (2) successive, adjoining, lateral sections of originally noninfested soil taken at progressively greater distances from infested soil, Manhattan, Kans.*

Series No.	Location and treatment of soil prior to testing	Tests	Plants	Average height of plants	Plants tillering	Culms			Plants dead	Diseased—			Roots retained	Disease severity			
						Total	Heading	Average per tillering plant		Crowns	Culms	Roots		Average on—			Final rating
														Crowns	Culms	Roots	
		Number	*Number*	*Inches*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pct.*	*Pct.*
1a	Naturally infested soil not cropped preceding year	4	41	10.5	15.0	53	0	2.3	38.9	100.0	61.1	96.5	60.0	68.9	57.7	62.5	67.7
1b	Sections of originally noninfested noncropped soil adjoining noncropped infested soil:																
	1–3 inches from noncropped infested soil	2	23	17.5	82.9	62	18.1	3.0	0	0	0	0	100.0	0	0	0	0
	4–6 inches from noncropped infested soil	2	23	17.0	75.7	49	20.6	2.5	0	0	0	0	100.0	0	0	0	0
	7–9 inches from noncropped infested soil	2	21	16.5	91.6	57	18.7	2.9	0	0	0	0	100.0	0	0	0	0
	10–12 inches from noncropped infested soil	2	20	17.5	80.0	49	17.2	2.8	0	0	0	0	100.0	0	0	0	0
2a	Naturally infested soil cropped to wheat preceding year	3	25	14.5	17.6	33	0	3.1	18.7	100.0	7.1	53.7	79.0	58.0	5.0	56.2	32.6
2b	Sections of originally noninfested noncropped soil adjoining cropped infested soil:																
	1–3 inches from cropped infested soil	2	23	22.0	24.2	33	59.0	2.4	0	96.1	0	25.0	94.5	13.5	0	37.5	13.5
	4–6 inches from cropped infested soil	2	22	27.5	38.3	34	60.3	2.5	0	50.0	0	2.5	100.0	1.0	0	1.0	4.4
	7–9 inches from cropped infested soil	2	21	24.5	65.9	41	53.2	2.5	0	50.0	0	2.5	100.0	1.0	0	1.0	3.5
	10–12 inches from cropped infested soil	2	21	28.0	61.3	54	43.1	2.8	0	100.0	0	5.0	100.0	2.0	0	3.5	8.1
3a	Naturally infested soil not cropped	3	25	16.0	18.4	33	3.7	2.0	8.3	100.0	41.6	83.7	68.7	61.2	37.0	65.6	44.8
3b	Sections of originally noninfested cropped soil adjoining noncropped infested soil:																
	1–3 inches from noncropped infested soil	2	18	22.0	50.0	35	24.0	3.1	10.0	100.0	67.7	93.9	58.8	65.3	17.0	75.0	47.1
	4–6 inches from noncropped infested soil	2	20	18.0	23.7	27	13.6	2.6	20.1	100.0	99.9	97.5	40.0	80.4	58.6	87.5	96.2
	7–9 inches from noncropped infested soil	2	21	27.5	46.3	35	65.0	2.5	0	100.0	0	57.5	97.5	5.0	0	5.0	11.4
	10–12 inches from noncropped infested soil	2	20	27.9	66.6	45	55.5	3.0	0	6.2	0	0	100.0	5.0	0	0	.9

TABLE 5.—*Vertical spread of Ophiobolus graminis in soil as judged by the severity of take-all in Kanred wheat grown in the greenhouse in samples of (1) infested soil and (2) successive horizontal, 1-inch layers above or below infested soil, Manhattan, Kans.*

Series No.	Location and treatment of soil prior to testing	Tests	Plants	Average height of plants	Plants tillering	Culms			Plants dead	Diseased—			Roots retained	Disease severity			
						Total	Heading	Average per tillering plant		Crowns	Culms	Roots		Average on			Final rating
														Crowns	Culms	Roots	
		Number	*Number*	*Inches*	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Pct.*	*Pct.*
4a	Naturally infested cropped soil from above noninfested soil	2	17	19.5	6.2	18.0	38.8	2.0	37.5	100.0	66.6	95.0	47.5	72.2	63.1	75.0	93.4
4b	Layers of originally noninfested soil below infested cropped soil:																
	First 1-inch layer below	2	21	29.5	70.0	34.0	44.0	2.0	0	9.5	0	1.0	100.0	1.0	0	3.0	.9
	Second 1-inch layer below	2	23	31.0	66.2	44.0	38.1	2.8	0	.5	0	.5	100.0	.5	0	.5	.1
	Third 1-inch layer below	2	25	30.0	63.7	62.0	41.8	3.4	0	0	0	0	100.0	0	0	0	0
	Fourth 1-inch layer below	2	20	31.0	80.0	44.0	60.4	2.5	0	0	0	0	100.0	0	0	0	0
	Fifth 1-inch layer below	2	22	29.5	60.0	42.2	59.5	2.5	0	0	0	0	100.0	0	0	0	0
5a	Infested soil below layers of noninfested soil	2	19	17.0	0	19.0	0	0	25.6	100.0	38.5	97.5	46.5	57.8	86.0	77.5	82.9
5b	Layers of originally noninfested, cropped soil above infested soil:																
	First 1-inch layer above	2	22	30.5	77.2	62.0	49.3	3.4	0	50.0	0	10.5	99.0	2.5	0	15.0	4.7
	Second 1-inch layer above	2	24	29.0	84.2	74.0	37.0	3.5	0	78.5	0	22.5	97.5	15.0	0	37.5	9.1
	Third 1-inch layer above	2	20	29.0	87.5	60.0	31.4	3.4	0	62.5	0	3.0	99.5	5.0	0	15.0	5.4
	Fourth 1-inch layer above	2	19	31.0	95.0	71.0	47.6	3.9	0	88.8	0	7.5	99.5	5.0	0	37.5	7.9
	Fifth 1-inch layer above	2	31	27.5	67.6	66.0	45.5	2.7	0	76.6	0	11.0	99.0	5.0	0	37.5	8.0

the field and greenhouse. In an experiment conducted in the greenhouse, stubble and roots from diseased plants grown in the field were mixed with sterilized soil at the rate of 1 part stubble to 3 parts soil by volume. In another experiment, small pieces of leaf sheath carrying the perithecia of *Ophiobolus graminis* were placed on the soil surface at the base of wheat plants in the heading stage. The plants were growing in sterilized soil. In a similar experiment in the field a 2- to 3-inch layer of infested stubble and roots was spaded into the top 6-inch layer of noninfested soil, after which wheat was planted.

In the greenhouse experiments, the plants became badly diseased the first year. All four replications gave the same result. The leaf sheaths with embedded perithecia caused 30.7 percent of the heads to become white. However, there were but few lesions apparent. The same soil later grew two badly diseased wheat crops. In the field experiments, no disease had appeared at the end of 2 years.

INFESTATION OF SOIL BY MEANS OF CULTURES ON ORGANIC MEDIA

Soil may be infested with *Ophiobolus graminis* by introducing into it an organic medium upon which the organism is growing. A sterilized mixture of barley and oat kernels, mixed in the ratio of half and half, has been used successfully. The infested material may be mixed with soil shortly before seeding or it may be placed in the soil adjacent to growing wheat plants at any stage of maturity. Usually, good infection results in the field and greenhouse. Especially heavy infection occurs on seedlings when the soil in which they are grown is infested in this way at seeding time. Within 15 days after emergence, many of the seedlings may be killed. However, when soils thus artificially infested were again seeded to wheat within 2 or 3 months very little infection occurred.

In a single experiment to determine the duration of infestation when accomplished in this manner, 3 parts of sterilized greenhouse soil were mixed with 1 part by volume of the infested medium. Wheat was grown to maturity in the greenhouse for 5 successive years and the crop was badly diseased each year. In a similar experiment in the field the medium was placed in trenches beside growing wheat plants and covered. Diseased plants were produced the first year, but because of unforeseen circumstances the field soil then had to be removed to the greenhouse. The first crop, after the soil had been removed to the greenhouse, was not infested. Abundant disease appeared, however, in succeeding years

DISCUSSION AND CONCLUSIONS

From the data presented, it appears possible that take-all may be carried by wind, water, farm implements, spores, the roots of living diseased wheat plants, and by dead diseased material. However, its spread from infested to noninfested soil in the field is a very slow process, fraught with many uncertainties. Even though an infested particle may be carried, it is not assured that infestation will ever increase, if at all, to a sufficient extent to cause disease, for heavily infested soil may lose its infestation in a short time when in contact with noninfested soil. In the greenhouse, where conditions are ideal for the disease, the process is more certain and is greatly speeded up

as compared with field conditions. Infestation has disappeared in the greenhouse also when the infested soil was in contact with non-infested soil.

It is rather common, in take-all-infested fields in central Kansas, for the disease to disappear from fields continuously cropped to wheat. For example, the wheat in a certain field was very severely diseased in 1923. This field was cropped to wheat continuously until 1934 without the reappearance of take-all. However, soil removed from this field to the greenhouse in 1930 produced wheat plants with considerable take-all. This also occurred in soil from other fields in which infestation had seemed to disappear.

The ease with which infestation may be secured from spores might appear to be somewhat inconsistent with the slow spread of the disease. This is accounted for by the fact that this fungus does not produce spores prolifically in the Middle West and that the spores when formed are released only under special conditions. Perithecia are seldom found unless the season is exceptionally moist. No doubt spores are an efficient means of spread where they occur, as pointed out by Samuel and Garrett [3] in South Australia. These writers also state that the spores live only 3 or 4 days when dry and that they are ejected under moist conditions only.

The evidence at hand seems to indicate that the most positive and the most frequently operative factor for the establishment, maintenance, and spread of *Ophiobolus graminis*, particularly in the Middle West, is the roots of the living host plant. This is in accord with results recently reported by Padwick,[4] Garrett,[5] and Adam and Colquhoun.[6] In all the greenhouse experiments performed by the writers in which the spread or establishment of infestation occurred, the roots of the host plant were present. In the experiments in which no spread took place, there were no growing wheat roots in the soil. It seems that the spread and establishment of the disease may well proceed in two steps: (1) It is spread as mycelium or spores by any of the mechanical or natural agencies that may be operating; and (2) its establishment in new locations depends largely on the presence of the living host plant.

Further evidence on this point has been presented by Sewell and Melchers,[7] who pointed out that crop rotation is an effective means of controlling take-all. In other words, soil infestation diminishes with the absence of the host plant.

The more or less circular outline of take-all spots in the field indicates uniform spread from a central point. Mycelium, if unobstructed when growing in a medium, forms a circular colony. The roots of a wheat plant are fairly equally distributed through the soil in all directions from the crown.

Consideration of the shape, orientation, and distribution of naturally infested spots in the field may supply information regarding the role of distributing agencies. Thus, if water run-off, farm implements, and

[3] SAMUEL, GEOFFREY, and GARRETT, S. D. ASCOSPORE DISCHARGE IN OPHIOBOLUS GRAMINIS, AND ITS PROBABLE RELATION TO THE DEVELOPMENT OF WHITEHEADS IN WHEAT. Phytopathology 23: 721–728. 1933.

[4] PADWICK, G. W. INFLUENCE OF WILD AND CULTIVATED PLANTS ON THE MULTIPLICATION, SURVIVAL AND SPREAD OF CEREAL ROOT-ROTTING FUNGI IN THE SOIL. Canad. Jour. Research 12: [575]–589. 1935.

[5] GARRETT, S. D. SOIL CONDITIONS AND THE TAKE-ALL DISEASE OF WHEAT. Ann. Appl. Biol. 23: 667–699. 1936.

[6] ADAM, D. B., and COLQUHOUN, T. T. THE SPREAD OF TAKE-ALL THROUGH THE SOIL. Jour. Aust. Inst. Agr. Sci. 2: 172–174. 1936.

[7] SEWELL, M. C., and MELCHERS, L. E. THE EFFECTS OF ROTATION AND TILLAGE ON FOOT-ROT OF WHEAT IN KANSAS, 1920–1924. Jour. Amer. Soc. Agron. 16: 768–771, illus. 1924.

wind are factors in distributing infested soil, it would be expected that the relative location and shape of infested areas might reflect this fact. Elongation and frequency of spots appear to have no relation to drainage, direction of prevailing winds, or direction of cultivation. Since the evidence shows that these may be agencies in distribution, the above observations emphasize the fact that it is difficult for the organism to establish itself in new locations.

Evidently *Ophiobolus graminis* can live for a period of years in soil cropped to wheat and produce no visible lesions, but if conditions become especially favorable for at least 2 successive years, plants growing in the soil may become infected.

SUMMARY

Experiments were conducted to determine whether *Ophiobolus graminis* may be distributed by the means ordinarily known to carry soil-borne parasites. The data indicate that any agency that can move soil particles or plant debris may carry infestation. However, the establishment of take-all in a new location is slow and uncertain.

In the greenhouse at least 25 percent, by volume, of infested soil in a mixture of infested and noninfested soil was required to produce appreciable disease on the succeeding wheat crop. Successive crops grown in the same mixture became increasingly more severely diseased. Infestation in a mixture containing only 15 percent of infested soil did not increase with 4 years' cropping. The concentration of infestation in either of these mixtures is much greater than would ordinarily result from the transfer of the soil by natural means.

Noninfested soil contaminated with a water suspension from infested soil produced infested wheat plants in the greenhouse when cropped with wheat for 2 years. Infection did not appear during 3 years' cropping in the field; but infection appeared when the soil was removed to the greenhouse and cropped for 2 more years.

Wheat plants, grown in noninfested soil that had been inoculated by applying infested soil lightly to the surface to simulate wind blowing, did not become diseased in the field during 3 years' cropping, but the disease appeared when this soil was brought to the greenhouse and cropped 2 more years.

Ophiobolus graminis did not spread from infested to noninfested soil when such soils were placed in contact without mixing and no wheat roots were growing through the adjacent sections, but when wheat roots were permitted to grow through these sections in either direction, the fungus spread to the noninfested soil. Such spread was greater when the wheat roots grew from the noninfested into the infested soil than when they grew from the infested into the noninfested soil. This was probably due to the greater growth of wheat roots in the noninfested soil.

Infested soils when placed in contact with noninfested soil often lost their infestation both in the field and in the greenhouse. Infestation often apparently disappears from take-all spots in the field.

Dead diseased host remains were a fairly good carrier of infestation in the greenhouse but not in the field.

Spores from the perithecia of *Ophiobolus graminis* were found to be an excellent source of infection, but they are seldom found in the Middle West.

Cultures of *Ophiobolus graminis* grown on a sterilized barley-oat medium were used successfully to infest soil in the field and greenhouse. The soils thus infested remained infested for a long period.

The infected roots of the living host plant are perhaps the most positive factor in carrying *Ophiobolus graminis* to noninfested soil and they contribute greatly to its establishment in a new location. This explains why rotation of crops is an effective control for take-all, particularly as it occurs in the Middle West.

THE FOREST FLOOR OF THE CHAPARRAL IN SAN GABRIEL MOUNTAINS, CALIF.[1]

By JOSEPH KITTREDGE, Jr.[2]
Forest ecologist, California Agricultural Experiment Station

INTRODUCTION

The forest floor in the chaparral of southern California as in forests of larger trees has a certain importance in maintaining the productivity of the soil and in such water relations as evaporation, surface run-off, and infiltration. The term "forest floor" is used to designate the total accumulation of organic materials above the mineral soil. The study reported herein was undertaken in an attempt to obtain quantitative measurements of the amount of floor material and its water relations.

REVIEW OF LITERATURE

Earlier determinations of the properties of forest floor from forest stands in the United States and Europe have been summarized by Alway and Zon (*2*).[3] However, none of them originated in the chaparral or its associated prolonged dry summer climate. Maximum dry weights per acre of 119.2 metric tons have been reported for a mixed forest of birch, maple, and spruce in New Hampshire (*8*), and of 87.5 metric tons for spruce, fir, and birch in northern Minnesota (*1*). Low values for well-stocked stands seem to be prevalent in the Southeast where records for mixed hardwoods and shortleaf pine range from 1 to 7 metric tons per acre (*6*, *11*) and in Florida, where undisturbed old-growth longleaf pine had only 1.7 metric tons per acre (*3*). In northeastern California, Bodman found 11 metric tons per acre under mature pine and 21 under white fir (*4*). In the mixed conifer forest of the west slope of the Sierra Nevada, 28 metric tons per acre was found by Lowdermilk (*7*). He also recorded 25 tons from the mixed "oak-chaparral" near Berkeley.

Volume weights of 0.26 for pine and from 0.19 to 0.31 for white fir floor were reported by Bodman (*4*).

From the same source, moisture content at cessation of movement ranged from 50 to 85 percent, corresponding to depth of water retained of from 0.15 to 0.3 inch. For the pine-fir floor of the Sierra Nevada, the normal or field moisture capacity averaged 180 percent or 0.26 inch depth of water (*7*). Values for forest-floor material from 100 to as high as 458 percent of the dry weight have been reported (*9*), but it is not certain that the higher figures do not represent moisture content nearer to saturation than to field moisture capacity.

[1] Received for publication May 2, 1938.
[2] The help of the following assistants at different times in field and laboratory is gratefully acknowledged: W. E. Davis, M. B. Dickerman, H. A. Fowells, P. G. Haddock, W. R. Howden, E. D. Marshall, K. A. Taber, and K. F. Thielking.
[3] Italic numbers in parentheses refer to Literature Cited, p. 534.

DESCRIPTION OF THE AREAS STUDIED

The field work was done on two areas on the headwaters of tributaries of Big Dalton and San Dimas Canyons in the San Gabriel Mountains of Los Angeles County. More specifically, one area of 240 acres included three small watersheds at the head of Bell Canyon at elevations of from 2,500 to 3,500 feet; and the other, with an area of 128 acres, three watersheds at the head of Fern Canyon at elevations of from 4,500 to 5,400 feet. These areas, originally called the "triplicate" watersheds, are being studied intensively by the California Forest and Range Experiment Station on the San Dimas Experimental Forest. The work here presented has been carried on with the cordial and helpful cooperation of the Station.[4]

In both areas, the topography is steep and mountainous with narrow V-shaped canyons and only minor development of small tributary drainageways. The slopes vary from 15° to 45° and are clothed with a rather shallow mantle of sandy loam to loam soil, in most places, with considerable admixture of gravel and stones.

Except for an occasional narrow strip of canyon bottom, the Bell Canyon watersheds were almost completely denuded of vegetation by the San Gabriel fire of 1919. Consequently, in 1933, when most of the field work was done, the area was occupied by an even-aged stand of chaparral, 14 years old, composed chiefly of chamiso (*Adenostoma fasciculatum*) and *Ceanothus crassifolius*. These species were important components of the chaparral before the fire and they are typical of large areas at similar elevations where the fire histories have varied widely. Fern Canyon had not been burned for more than 55 years, although fire scars on the occasional larger oaks and bigcone spruces testify to earlier fires. The north and east slopes are clothed with a rather dense stand of canyon live oak (*Quercus chrysolepis*) of sprout origin and the south and west slopes with a mixture of manzanita (chiefly *Arctostaphylos glauca*) and *C. divaricatus*.

The sampling points were distributed mechanically over each area with the intention of obtaining a representation from which, by biometric analyses, inferences might be drawn for the area as a whole. Eighty-four sampling points were studied in Bell Canyon and 101 in Fern. At each one the percentage of crown density or coverage was estimated by species on an area of 1 milacre, and the average height and crown or stem diameter of each species was recorded.

A total of 12 species in Bell Canyon and 7 in Fern Canyon made up the principal woody components, various combinations of which formed the chaparral communities or types. These were segregated and characterized by the one or two predominating species and then combined in broader groups if differences between the types were found not to be significant. The first analysis by types, therefore, included 14 communities in Bell and 11 in Fern Canyon.

These differences in the vegetation within either Bell or Fern Canyon might be expected to be reflected in differences in the floor and its water relations. However, differences between Fern and Bell Canyon, which are separated by about 6 miles and some 2,000 feet in elevation, are doubtless associated with differences in soil and climate as well as with differences in vegetation.

[4] The work was made possible by the allotment of funds from a special State appropriation for research in forestry in cooperation with Federal agencies.

COVERAGE AND DEPTH OF FOREST FLOOR AND CROWN DENSITY

The forest floor, or A_0 horizon, was found to cover from 0.4 to 0.9 of the surface in Bell Canyon and from 0.6 to 1.0 in Fern Canyon, in each case with only three or four exceptions provided by bare spots.

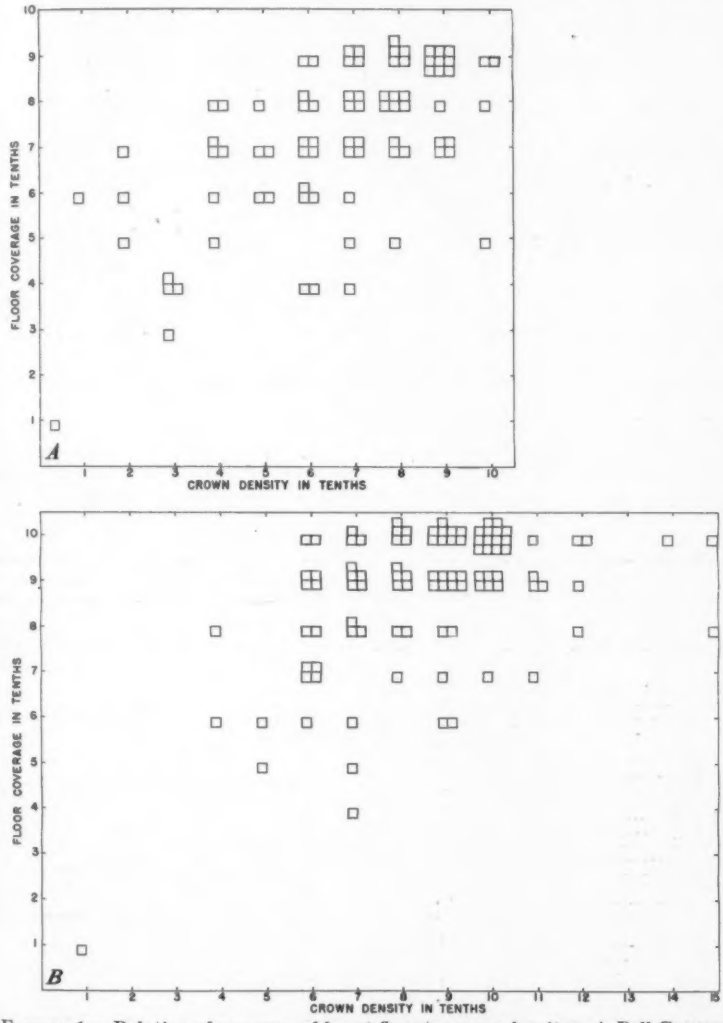

FIGURE 1.—Relation of coverage of forest floor to crown density: *A*, Bell Canyon; *B*, Fern Canyon. Each small square represents one sample.

The distribution of the coverage by the forest floor in relation to the crown density, also expressed on a scale of tenths, is shown in figure 1 for Bell and Fern Canyons. In general the proportion of area covered by

the forest floor is somewhat greater than that covered by the crowns. In Bell Canyon, on an average, a floor coverage of 0.8 is associated with a crown density of 0.7, and in Fern Canyon the corresponding figures are 0.9 and 0.8.

In depth the floor varied from 0 to 1 inch, with an average of 0.25 inch in Bell, and up to 2 inches in Fern Canyon, with an average of about 0.6 inch.

AMOUNT OF FOREST FLOOR IN DRY WEIGHT PER ACRE

The amount of the forest floor was determined by collecting samples 1 square foot in area including all the organic accumulation down to the mineral soil, avoiding as far as possible the mineral matter. However, on the steep slopes where there is considerable creep, the separation is difficult to make and samples inevitably include mineral fragments. These were separated by sieving and floating the material in the laboratory; the remaining organic matter was oven-dried for 132 hours at 70° to 80° C.[5] The resulting weights converted to an area of 1 acre will be termed "dry weight per acre."

These dry weights of single samples varied from 0.0 to 20.4 metric tons per acre for Bell Canyon, and up to 37.6 for Fern Canyon. The averages by chaparral types ranging from 2.9 to 21.2 metric tons per acre are shown in tables 1 and 2. The figures for number of samples show the distribution of sampling points by types and, since the sampling points were mechanically spaced, they give an indication of the relative abundance of the different types. Thus the types that occupy only small areas are represented by only a few samples and the averages for these less extensive types are correspondingly less reliable.

TABLE 1.—*Dry weight of forest floor in Bell Canyon*

Group and chaparral type[1]	Means of dry weight per acre	Standard error	Coefficient of variation	Samples	Significance of differences between means[2]				
					Sm-Eff	Eff	Qd-Cer	Af-Cer	Af-Sm
	Metric tons	*Metric tons*	*Percent*	*Number*					
High group:									
Ro	12.33	4.02	45.9	2	s	s	n	hs	n
Gv	9.50			1					
Sm-Ccr	7.40	1.72	52.0	5	n	n	n	s	n
Pa	7.22	1.29	30.8	3	s	n	n	n	n
Ccr-Sm	6.72	1.71	84.3	11	n	n	n	n	n
Cb	6.23	3.92	88.8	2					
Co-Qwf	5.74	2.92	71.8	2					
Medium group:									
Ccr-Af	5.17	1.02	89.5	21					
Af-Sm	4.82	1.47	74.3	6					
Af-Ccr	4.21	.58	55.4	16					
Ag	4.13			1					
Qd-Ccr	3.75	1.14	60.2	4					
Low group:									
Eff	3.27	.80	54.1	5	Medium and low		Low		Medium
Sm-Eff	2.92	.85	65.0	5					
Group means:									
High	7.33	1.00	69.2	26	hs		s		s
Medium	4.67	.53	78.5	48	n		n		n
Low	3.10	.59	59.6	10					
Medium and low	4.40	1.01	79.1	58					
All types	5.31	.46	79.3	84					

[1] Abbreviations as here used are as follows: Af, *Adenostoma fasciculatum*, chamiso; Ag, *Arctostaphylos glauca*, manzanita; Cb, *Cercocarpus betuloides*, mountain-mahogany; Ccr, *Ceanothus crassifolius*,; Co, *C. oliganthus*, hairy ceanothus; Eff, *Eriogonum fasciculatum* var. *foliolosum*, wild buckwheat; Gv, *Garrya veatchii*, silktassel; Pa *Photinia arbutifolia*, christmasberry; Qd, *Quercus dumosa*, scrub oak; Qwf, *Q. wislizenii* var. *frutescens* interior live oak; Ro, *Rhus ovata*, sugar sumac; Sm, *Salvia mellifera*, black sage.

[2] s=significant; hs=highly significant; n=not significant.

[5] The dry weights thus obtained were found by test to be from 0.3 to 0.9 percent higher than those in an oven at 105° C. The lower temperature was used to avoid losses by combustion.

TABLE 2.—*Dry weight of forest floor in Fern Canyon*

Group and chaparral type [1]	Means of dry weight per acre	Standard error	Coefficient of variation	Samples	Significance of differences between means [2]				
					Pm	Qc	Qwf	Qc-Qwf	Qc-Ag
	Metric tons	*Metric tons*	*Percent*	*Number*					
High group:									
Ag [1]	21.18	3.70	49.4	8	n	h s	n	n	
Ag-Af	20.56	3.89	42.3	5	n	s	n	n	
Ag-Cd	20.16	1.84	32.8	13	s	h s	s	s	n
Cd-Ag	19.74	2.93	33.2	5	s	n	n	n	
Medium group:									
Qwf-Ag	17.57	4.02	51.1	5	------	n			
Qc-Ag	15.42	3.53	56.1	6					
Low group:									
Qc-Qwf	12.84	2.21	45.6	7					
Qwf	12.29	1.72	36.9	7					
Qc	12.24	3.76	67.6	40	Low	Medium			
Pm	10.11	1.50	25.3	4					
Af	4.69	----------	----------	1					
Group means:									
High	20.42	1.46	39.8	31	h s	n			
High and medium	19.37	1.31	43.9	42	h s				
Medium	16.40	2.67	54.1	11	n				
Low	12.04	.90	58.2	59					
All types	15.09	.84	56.4	101					

[1] Abbreviations used: Ag, *Arctostaphylos glauca*, manzanita; Af, *Adenostoma fasciculatum*, chamiso; Qc, *Quercus chrysolepis*, canyon live oak; Cd, *Ceanothus divaricatus*; Qwf, *Quercus wislizenii* var. *frutescens*, interior live oak; Pm, *Pseudotsuga macrocarpa*, bigcone-spruce.

[2] See footnote 2, table 1.

The point that stands out in these figures is that the differences in the average weight of floor in different types are small. These differences were tested for significance by the *F* test in the analysis of variance as outlined by Snedecor (*12*), and the expectation was verified that many of the types are not significantly different one from another in average dry weight of floor per acre. The significance of the difference is indicated in the right-hand columns of the tables, where the types or groups from the low end of the series are repeated as headings of the columns to form a cross classification with the types or groups of the high end of the series in the left-hand column. On that basis the types were combined into three groups for each of the two canyons in such a way that differences between groups would be significant and, at the same time, the groups would include types that seemed to have some logical interrelation.

The 12 species that are commonly represented in Bell Canyon and give character to the chaparral communities occur in various mixtures in which the relations to site or to natural succession are not well defined. Almost all the species were included by Cooper (*5*) as components of the chaparral climax. If they have successional relations, the 14 years since the last fire has not been sufficient for their expression. It seems likely that the *Rhus* and *Garrya* and *Photinia*, which have heavy accumulations of forest floor, may represent more persistent species in succession than the *Eriogonum* and chamiso (Af), which have light forest floors. Actually, by the test of significance, the *Rhus* with an average of 12.33 metric tons per acre is significantly different from the *Eriogonum* types with less than 3.3 metric tons, and from the chamiso—*Ceanothus crassifolius* type with 4.21 metric tons. This latter type is also significantly different from the type in which the black sage (Sm) is dominant in association with the *Ceanothus* and in which the forest floor averages 7.4 metric

tons. The only other case of a significant difference between means is that of Christmasberry (Pa) with 7.22 metric tons, as compared with the black sage and *Eriogonum* with 2.92 metric tons. Obviously there is little justification for distinguishing the 14 types on the basis of the amount of forest floor.

When they are combined in three groups, some of the homogeneity of the individual types is lost, with a compensating gain in differentiation. The low group, with an average dry weight of forest floor of 3.1 metric tons, is characterized by the *Eriogonum;* the intermediate group, with 4.67 metric tons, contains the types with chamiso; and the high group, with 7.33 metric tons, a variety of types including those which have been suggested as more persistent. Between the means of these three groups, the high is significantly different from both the medium and the low, but the intermediate group is not significantly different from the low. Going a step further, therefore, and combining the intermediate and low groups, with further loss of homogeneity, there remains a high group with a mean dry weight of 7.33, which is significantly different with a probability of 100 to 1 from the combined low group with a mean of 4.4 metric tons per acre.

The variation within the types and in the groups is high throughout, as indicated by the magnitudes of the coefficients of variation, which range from 30.8 to 89.5 percent. The means of the two groups, high and medium and low, have standard errors of 1 metric ton per acre, which may be interpreted by saying that another sample of the same size from watersheds with similar vegetation would give means for the high group ranging between 6.3 and 8.3 and for the low group between 3.4 and 5.4 metric tons, with a probability of 68 in 100.

In Fern Canyon at elevations between 4,500 and 5,400 feet, the means for the forest floor in the different types range from 4.7 metric tons for chamiso (Af) to 21.18 for manzanita (Ag). The four types in which manzanita occurs pure or in mixture with chamiso or *Ceanothus divaricatus* are higher, and usually significantly higher, in dry weight of floor than the types characterized by oak or spruce or chamiso. If three groups of types are distinguished, only the high group of manzanita types is distinct from the low group with the oak (Qc, Qwf), bigcone-spruce (Pm), and chamiso. If the two types in which manzanita and oak are associated and the other types in which manzanita is present are combined, the resulting group with a mean weight of forest floor of 19.37 metric tons differs with high significance from the low group with 12.04 metric tons.

Again the variation within types and groups is high, ranging from about 25 to 68 percent and the standard errors of the group means, when only two groups are distinguished, are 1.3 metric tons for the high and medium and 0.9 for the low group. In this case, however, these two groups, the high one characterized by manzanita and the low one by oak or spruce, are quite definitely associated with a difference in site. The former is found on the south and west aspects, while the latter occurs on the north and east aspects and in the canyon bottoms.

When the 14-year-old chaparral of Bell Canyon below 4,000 feet is compared with the more than 50-year-old vegetation of Fern Canyon above 4,000 feet, the difference between 5.3 and 15.0 metric tons per acre dry weight of forest floor is highly significant.

An attempt was made to investigate the relation of the amount of forest floor to the foliage volume. As an expression of foliage volume, the product of the average height of the vegetation in feet and the crown density in tenths was used. This gave a scale ranging from less than 1 to 35. In Bell Canyon, where the foliage or crown length closely approached the height of the chaparral, there was a poorly defined tendency for the dry weight of forest floor to increase in a somewhat linear trend as the foliage volume increased. For separate groups of the chaparral types, however, the trend disappeared. In Fern Canyon, nothing that could be called a trend was found, perhaps because, with the oaks particularly, the total height was not a good index of crown length. In any case, no evidence was found of a usable relation between dry weight of forest floor and foliage volume.

VOLUME WEIGHT OF FOREST FLOOR

The volume weight of the forest floor, determined as the ratio of the oven-dry weight to the weight of an equal volume of water, provides a measure of the density of the floor material and might be expected to reflect differences in the moisture relations or in the composition of the chaparral as it influences the underlying mineral soil. The term "volume weight" is used as a synonym for "apparent specific gravity." The volume weights were obtained from the dry weights and the measurement of average depth of the square-foot samples from which volumes were computed. The average volume weights of the different chaparral types in Bell and Fern Canyons, together with the biometric analysis, are shown in tables 3 and 4.

TABLE 3.—*Volume weights of forest floor in Bell Canyon* [1]

Group and chaparral type	Volume weight	Standard error	Coefficient of variation	Samples	Significance of differences between means				
					Cb	Qd–Cer	Co–Qwf	Sm–Eff	Cer–Sm
High group:			*Percent*	*Number*					
Pa	0.27	0.02	13.6	3	s	n	s	n	n
Af–Sm	.26	.05	48.4	6	n	n	n	n	
Ro	.26	.004	2.8	2	s	n	s	n	
Sm–Cer	.25	.03	25.6	5	s	n	n		
Af–Cer	.24	.03	43.7	15	n	n			
Medium group:									
Eff	.23	.02	13.9	4	s	n	n		
Cer–Af	.22	.01	50.9	21					
Cer–Sm	.20	.03	58.3	11					
Sm–Eff	.18	.05	61.6	5					
Low group:									
Co–Qwf	.16	.02	22.6	2					
Qd–Cer	.15	.05	69.5	4					
Gv	.09			1	Low	Medium			
Cb	.08	.02	38.1	2					
Ag	.08			1					
Group means:									
High	.25	.02	38.9	31	h s	n			
Medium	.21	.02	51.4	41	s				
Low	.13	.02	61.1	10					
All types	.21	.01	49.5	82					

[1] For meaning of abbreviations see footnotes, table 1.

In Bell Canyon the range is from 0.08 to 0.27. The differences in the means between different types are small and consequently only a few of the higher ones are significantly different from the lower ones. Such significant differences as are found are between the less extensive types, such as Christmasberry (*Pa*) or *Rhus*, with volume weights of

TABLE 4.—*Volume weights of forest floor in Fern Canyon* [1]

Group and chaparral type	Volume weight	Standard error	Coefficient of variation	Samples	Significance of differences between means					
					Qc–Ag	Qwf–Ag	Qc	Qwf	Qc–Qwf	Ag
High group:			*Percent*	*Number*						
Af	0.36			1						
Ag–Cd	.29	0.02	20.4	13	h s	h s	h s	h s	s	n
Ag–Af	.28	.01	7.2	5	h s	h s	s	n		
Cd–Ag	.27	.03	27.4	5	s	n	n			
Pm	.25	.02	17.9	4	s	n	n			
Ag	.25	.02	25.2	8	s	n				
Low group:										
Qc–Qwf	.21	.03	42.0	7						
Qwf	.20	.03	35.5	7						
Qc	.19	.01	42.7	39						
Qwf–Ag	.18	.02	27.7	5						
Qc–Ag	.17	.02	28.4	6						
Group means:					Low					
High	.27	.01	22.6	36	h s					
Low	.19	.01	40.4	64						
All types	.22	.01	36.9	100						

[1] For meaning of abbreviations see footnotes, tables 1 and 2.

0.27 and 0.26, and *Cercocarpus betuloides* (Cb) or *Ceanothus oliganthus* and oak (Co–Qwf), with volume weights of 0.08 and 0.16.

The sequence of the types in descending order of the mean volume weights is not the same as was found in the analyses of dry weight or of percentage field moisture capacity or of depth of water retained. Correspondingly, the groups that can be formed on the basis of volume weight are not the same as those distinguished in the other analyses. However, if the groups on any other basis were used, there would be neither homogeneity within nor significant differences between them. It seems better, therefore, to differentiate the groups independently for volume weight; and for that purpose three groups designated "high," "medium," and "low" are distinguished. The high group, with mean volume weight of 0.25, coefficient of variation of 38.9 percent, and standard error of 0.02, contains the types in which chamiso (Af) predominates, together with Christmasberry, *Rhus*, and the black sage (Sm) with admixture of *Ceanothus crassifolius*. The intermediate group includes the two types in which *C. crassifolius* is dominant and the two in which *Eriogonum* is found. This group has a mean volume weight of 0.21, coefficient of variation of 51 percent, and standard error of 0.02. The low group includes the two types in which oak (Qd, Qwf) is an element, together with the *Garrya*, manzanita (Ag), and *Cercocarpus betuloides*.

As between these groups, the low is different from the high group with high significance, and is also significantly different from the medium group. The high and medium groups are not significantly different one from another. The coefficients of variation expressing the dispersion of the individual samples making up a type or group, and the standard errors representing the value of the means for purposes of generalization or prediction are not very different for the groups from their values for the types. In other words, the subdivision of the vegetation into a larger number of types does not increase either the homogeneity of the units or the value of the means for application to larger areas of similar vegetation.

In Fern Canyon, the volume weights for the chaparral types vary from 0.17 to 0.36. In general the variation within types is less than

was the case in Bell Canyon, as might be anticipated from the fact that the types in Fern Canyon have had an opportunity to develop characteristic properties undisturbed over a much longer period of time. Although the range of the mean volume weights and the differences between individual types are not greater than in Bell Canyon, the types with high volume weight are in many cases significantly different from those with low. Thus the types with manzanita (Ag) are significantly different, and in several cases with probabilities of more than 99 in 100, from the types in which either oak (Qc, Qwf) predominates.

These types on the bases of biometric analysis, composition, and site fall rather definitely into two groups. The high group contains the mixtures of manzanita, chamiso (Af), and *Ceanothus divaricatus*, together with the bigcone-spruce (Pm). This group has a mean volume weight of 0.27 with a coefficient of variation of 22.6 percent and standard error of 0.01. The low group contains all of the oak types and their mixtures and has a mean volume weight of 0.19 with a coefficient of variation of 40.4 percent and a standard error of 0.01. The satisfactory basis for the separation of these two groups is confirmed by the finding that the difference between their means is highly significant.

The mean volume weight of 0.21 for all of the samples from Bell Canyon is not significantly different from the closely corresponding value of 0.22 for Fern Canyon.

FIELD MOISTURE CAPACITY

The field moisture capacity of the floor samples was determined in the laboratory by a modification of the method proposed by Shaw (*10*). After the mineral matter had been separated and removed, the sample was placed in a metal cylinder and immersed in water for 48 hours. The cylinders were then removed and allowed to drain for a few minutes until the freely flowing water had drained off. They were then placed on boxes of dry loam soil, the floor material being separated from the mineral soil of the boxes by a layer of cheesecloth. The tops of the cylinders were sealed with waxed cloths to prevent loss of moisture by evaporation. The samples were left to drain in this way for 48 hours, when the floor was removed and all or a portion of it dried as a basis for expressing the field moisture capacity as a percentage of the oven-dry weight.

This method was found to give results not reproducible within about 10 percent. Tests were made of the effect of resoaking and running samples a second time, of using a sandy loam in comparison with the loam soil, of setting only one instead of three cylinders in a single soil box, and of rearranging the forest-floor material in the cylinders. None of these tests showed any consistent effect on the amount of moisture retained, or on the percentage of field moisture capacity. It seems reasonable to conclude, therefore, that, although individual determinations are not closely reproducible, the means of several determinations within a type or group are reliable as indicating the retention of capillary moisture by the floor.

As in the case of dry weights, the field moisture capacity was first investigated for the individual chaparral types. The figures are shown in tables 5 and 6.

TABLE 5.—*Field moisture capacity of forest floor in Bell Canyon* [1]

Group and chaparral type	Mean field moisture capacity	Standard error	Coefficient of variation	Samples	Significance of differences between means			
					Cer–Af	Af–Cer	Cer–Sm	Af–Sm
High group:	*Percent*	*Percent*	*Percent*	*Number*				
Co-Qwf	191	18	14	2	h s	h s	n	n
Eff	190	18	19	4	h s	h s	n	n
Ro	178	5	4	2	s	s	n	n
Medium group:								
Qd-Cer	165	21	25	4	n	n	n	n
Pa	164	11	11	3				
Cb	162	26	22	2				
Sm-Cer	161	7	10	5				
Sm-Eff	159	8	11	5				
Af-Sm	147	10	16	6				
Cer-Sm	145	11	26	11				
Low group:								
Gv	140			1				
Af-Cer	139	5	13	15				
Cer-Af	139	4	14	21				
Ag	125			1				
					Low	Medium		
Group means:								
High	187	10	16	8	h s	s		
Medium	154	5	20	36	s			
Low	139	3	13	38				
All types	150	3	20	82				

[1] For meaning of abbreviations see footnotes, table 1.

TABLE 6.—*Field moisture capacity of forest floor in Fern Canyon* [1]

Group and chaparral type	Mean field moisture capacity	Standard error	Coefficient of variation	Samples	Significance of differences between means					
					Ag-Cd	Ag-Af	Cd-Ag	Ag	Qwf-Ag	Qc-Ag
High group:	*Percent*	*Percent*	*Percent*	*Number*						
Qc	173	4	15	40	h s	h s	s	s	s	n
Qc-Qwf	171	10	15	7	h s	s	s	n	n	
Pm	167	16	19	4	s	n	n	n		
Medium group:										
Qwf	158	12	19	7	s	n	n	n		
Qc-Ag	154	15	25	6	n	n	n	n		
Qwf-Ag	148	8	12	5						
Low group:										
Ag	144	9	18	8						
Cd-Ag	138	6	10	5						
Af	138			1						
Ag-Af	135	8	14	5						
Ag-Cd	130	5	15	13						
					Low	Medium				
Group means:										
High	172	4	16	51	h s	s				
Medium	154	7	20	18	s					
Low	136	4	15	32						
All types	157	3	19	101						

[1] For meaning of abbreviations see footnotes, tables 1 and 2.

In general, the two predominating types in Bell Canyon, composed of mixtures of chamiso (Af) and *Ceanothus crassifolius*, and the four types with strong admixtures of black sage (Sm), tend to have a low field moisture capacity, that is, about 140 percent of the dry weight; whereas the oak (Qd, Qwf), *Eriogonum*, *Rhus*, and Christmasberry (Pa) have high average percentages ranging from 164 to 191. The total range for the types in Bell Canyon is only from 125 to 191 per-

cent. The standard errors of the means range from 4 to 26 percent. The coefficients of variation for the individual types, ranging from 4 to 26 percent, are fairly low. This is in contrast to the high variability which was found for the dry weights and other properties. Considering the limited range and the small differences between individual types, it is not surprising that most of them are not significantly different, one from another. Only the types with chamiso and *C. crassifolius* at the low end are significantly different from the *C. oliganthus*-oak, *Eriogonum*, and *Rhus* types at the high end.

When the three last-named types are combined in a group with an average field moisture capacity of 187 percent, this group is significantly different from the low group containing the chamiso-*Ceanothus crassifolius*, and single samples of the manzanita (Ag) and *Garrya* types, and also from the intermediate group containing the seven other types. These groups have coefficients of variation from 13 to 20 percent and standard errors from 3 to 10 percent.

Between the field moisture capacities and the amounts of forest floor, there is no definite relation either in the individual types or in the composition of the groups.

In Fern Canyon, the range of the means of field moisture capacity for individual types is from 130 to 173 percent. The five types at the lower end are those which have no oak and in which manzanita (Ag) is an important element, usually in mixture with chamiso (Af) or *Ceanothus divaricatus*. Canyon live oak (Qc) heads the list with 173 percent, and the mixture of canyon and interior live oaks (Qwf) and bigcone-spruce (Pm) are the next two in order. Notwithstanding the small range and small differences between mean field moisture capacities of the different types, the canyon live oak and spruce types are significantly different from most of the manzanita types. The coefficients of variation for individual types are again low, ranging from 10 to 25 percent and the standard errors of the means from 4 to 16 percent.

The types may be combined in three groups, a high group with canyon live oak or bigcone-spruce and without manzanita, with an average field moisture capacity of 172 percent; a low group, including four manzanita types without oak, having a field moisture capacity of 136 percent; and an intermediate group in which interior live oak or the mixture of oak and manzanita are represented, with a mean field moisture capacity of 154 percent. These three groups are significantly different, one from another. The coefficients of variation are from 15 to 20 percent; and the standard errors, from 4 to 7 percent.

In this instance of Fern Canyon, it is evident that the field moisture capacities of the different types tend to be inversely proportional to the dry weights of the forest floor; that is, the manzanita types which have high weight of floor have low field moisture capacities; and the oak and bigcone-spruce types which have low weights of forest floor tend to have high field moisture capacities.

When the forest floors of Fern and Bell Canyons are compared, the field moisture capacities are not significantly different.

DEPTH OF WATER RETAINED

If the weights of the forest-floor samples are multiplied by the percentages of water retained at field moisture capacity, and the product divided by a suitable constant, figures are obtained for the

inches depth of water retained by the forest floor at field moisture capacity on the basis of oven-dry weight. The results of these computations, together with the biometric analysis, are shown for the different chaparral types and groups for Bell Canyon in table 7 and for Fern Canyon in table 8.

TABLE 7.—*Depth of water retained by forest floor at field moisture capacity in Bell Canyon*[1]

Group and chaparral type	Water at field moisture capacity	Standard error	Coefficient of variation	Samples	Significance of differences between means				
					Sm-Eff	Qd-Cer	Af-Cer	Eff	Cer-Af
High group:	*Inches*	*Inches*	*Percent*	*Number*					
Ro	0.21	0.06	43	2	n	n	s	n	n
Gv	.13			1					
Sm-Cer	.12	.03	61	5	n	n	s	n	n
Cb	.12	.08	93	2	n	n	n	n	
Co-Qwf	.12	.07	78	2	n	n	n	n	
Pa	.11	.02	28	3	s	n	s	n	
Cer-Sm	.10	.02	79	11			n		
Low group:									
Af-Sm	.07	.03	82	6					
Cer-Af	.07	.02	93	21					
Eff	.06	.02	60	5					
Af-Cer	.06	.01	60	15					
Qd-Cer	.06	.02	57	4					
Ag	.05			1	Low				
Sm-Eff	.04	.01	63	5	hs.				
Group means:									
High	.12	.02	75	26					
Low	.06	.01	83	57					
All types	.08	.01	87	83					

[1] For meaning of abbreviations see footnotes to table 1.

TABLE 8.—*Depth of water retained by forest floor at field moisture capacity in Fern Canyon*[1]

Group and chaparral type	Water at field moisture capacity	Standard error	Coefficient of variation	Samples	Significance of differences between means		
					Pm	Qwf	Qc
High group:	*Inches*	*Inches*	*Percent*	*Number*			
Ag	0.29	0.04	43	8	n	n	n
Cd-Ag	.26	.04	33	5	n	n	
Ag-Af	.26	.05	40	5	n	n	
Qwf-Ag	.26	.07	62	5	n		
Qc-Ag	.26	.08	74	6			
Ag-Cd	.25	.03	42	13			
Low group:							
Qc-Qwf	.21	.04	50	7			
Qc	.20	.02	62	40			
Qwf	.18	.03	38	7			
Pm	.16	.02	24	4			
Af	.06			1	Low		
Group means:							
High	.26	.02	47	42	s		
Low	.19	.02	59	59			
All types	.22	.01	55	101			

[1] For meaning of abbreviations see footnotes to tables 1 and 2.

Because the types with the larger amounts of forest floor tend to have lower field moisture capacities and vice versa, the figures for depth of water retained, which integrate the two, would be expected to be less definitely differentiated. This proved to be the case. In

Bell Canyon the range of the means of the different types is from 0.04 to 0.21 inch. Only the mixed type of chamiso (Af) and *Ceanothus crassifolius*, and in one case the black sage (Sm)–Eriogonum mixture less common types are significantly different from certain of the black sage (Sm) mixtures.

When the types in Bell Canyon are grouped, a high group of seven types is found to be identical with the high group that was distinguished on the basis of dry weights of forest floor. The other seven types, including those with chamiso (Af) and *Eriogonum*, comprise a rather homogeneous group, characterized by low values for depth of water retained. The mean for the high group is 0.12 inch and for the low group 0.06 inch, and these two groups differ one from another with high significance.

The analysis shows somewhat similar results in Fern Canyon, except that the depth of water retained by the different types is greater with only two exceptions. The range, excluding a single sample of chamiso, is from 0.16 to 0.29 inch. The individual types are not significantly different one from another.

When the types in Fern Canyon are grouped, those with low values for mean depth of water retained form the same group of five types without manzanita which comprised the low group for dry weight of forest floor. The other six types containing manzanita (Ag) form a group with high values. The mean for this high group is 0.26 inch and for the low group 0.19, and the two groups are significantly different one from another.

There is almost no overlapping in the mean values of individual types between Bell and Fern Canyons, and the means for the respective watersheds are significantly different.

These figures for depth of water retained at field moisture capacity on the basis of oven-dry weight have special interest because they represent the depth of precipitation which would be retained by the forest floor, subject to evaporation into the air, and which would therefore not be available either for the growth of vegetation or for replenishing soil moisture or ground water. However, to use them in this way, a correction must be made because the forest floor under natural conditions is never reduced to an oven-dry condition. During dry weather the floor usually contains about 15 percent moisture content on the basis of oven-dry weight. Hence the figures for field moisture capacity in percentage would be reduced by 15 percent, or by the percentage of actual moisture content at the beginning of any rain, and the figures for depth of water retained could be reduced correspondingly. In light rains the forest floor may be a factor of some importance in intercepting moisture which would otherwise reach the soil. In heavy rains, on the other hand, it will affect only a negligible part of the total rainfall.

SUMMARY

The forest floor under the chaparral covers a large part of the surface within 15 years after a heavy burn.

The amount of forest floor varies widely within any given type of chaparral, although differences in means between types are small and only significant between the extremes. The differences become sig-

nificant if the chaparral is segregated into only two or three broad groups such as those characterized by chamiso (Af), manzanita (Ag), or oak (Qc, Qwf). The 15 metric tons average for Fern Canyon is almost three times that for Bell Canyon.

The mean volume weights for individual types vary from less than 0.1 to 0.36. In Fern Canyon there is a well-defined differentiation between the types or group with high means in which manzanita, chamiso, or bigcone spruce (Pm) predominate, and those with low means in which the oaks predominate.

Field moisture capacities vary less within types than the other measures. Three groups within each canyon show a significant difference in the means, although the difference between 157 percent in one canyon and 150 percent in the other is not significant. In general the manzanita, *Ceanothus*, and chamiso are associated with low field moisture capacities of the floor, and oak and big cone spruce with high.

The depth of water retained by the forest floor represents an integration of amount, volume weight, and field moisture capacity of the materials, and gives an indication of the role of the forest floor in intercepting rainfall. Like its component elements, it varies within types to such an extent that the small differences between the means of types are rarely significant. The groups of types, however, are distinct, a low group in Bell Canyon retaining 0.06 inch, a high group, 0.12 inch; and in Fern Canyon, a low group, 0.19, and a high group, 0.26 inch. These amounts, less the actual moisture content at the beginning of rain, would be held subject to evaporation from the forest floor and would not contribute to soil moisture or to surface run-off.

LITERATURE CITED

(1) Alway, Frederick J., and Harmer, Paul M.
1927. Minnesota glacial soil studies: I. The forest floor on the late Wisconsin drift. Soil Sci. 23: 57–71, illus.

(2) ——— and Zon, Raphael.
1930. Quantity and nutrient contents of pine leaf litter. Jour. Forestry 28: 715–727.

(3) Barnette, R. M., and Hester, J. B.
1930. Effect of burning upon the accumulation of organic matter in forest soils. Soil Sci. 29: 281–284, illus.

(4) Bodman, G. B.
1935. The forest floor developed under conditions of summer rainfall deficiency in a California pine and fir forest. Amer. Soil Survey Assoc. Bull. 16: 97–101, illus.

(5) Cooper, William S.
1922. The broad sclerophyll vegetation of California; an ecological study of the chaparral and its related communities. 124 pp., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 319.)

(6) Hursh, C. R.
1928. Litter keeps forest soil productive. South. Lumberman 133: 219–221.

(7) Lowdermilk, W. C.
1930. Influence of forest litter on run-off, percolation, and erosion. Jour. Forestry 28: 474–491.

(8) Lunt, Herbert A.
1932. Profile characteristics of New England forest soils. Conn. (State) Agr. Expt. Sta. Bull. 342, pp. 743–836, illus.

(9) MUNNS, E. N., and SIMS, IVAN H., compilers.
1936. FORESTS IN FLOOD CONTROL. [U. S.] 74th Cong., 2d sess., Com. on Flood Control, Sup. Rept. on H. R. 12517. 70 pp.
(10) SHAW, CHARLES F.
1927. THE NORMAL MOISTURE CAPACITY OF SOILS. Soil Sci. 23: 303–317, illus.
(11) SIMS, I. H.
1932. LITTER DEPOSITION AND ACCUMULATION IN THE PINE-OAK TYPE OF THE SOUTHERN APPALACHIANS. Jour. Forestry 30: 90–91.
(12) SNEDECOR, GEORGE W.
1934. CALCULATION AND INTERPRETATION OF ANALYSIS OF VARIANCE AND COVARIANCE. 96 pp. Ames, Iowa (Iowa State Col., Div. Indus. Sci. Monog. 1).

THE ANNUAL ACCUMULATION AND CREEP OF LITTER AND OTHER SURFACE MATERIALS IN THE CHAPARRAL OF THE SAN GABRIEL MOUNTAINS, CALIF.[1]

By JOSEPH KITTREDGE, Jr.

Forest ecologist, California Agricultural Experiment Station

The amount of organic material which accumulates each year beneath the chaparral in southern California constitutes one factor in the development of the forest floor, or A_0 horizon; and to the extent that it is composed of leaves, it may be an index of the transpiration of water by the vegetation. The annual accumulation has been collected each year for 1935, 1936, and 1937 from the "triplicate" watersheds at the heads of Bell and Fern Canyons in the San Dimas Experimental Forest of the San Gabriel Mountains.[2] The areas have been described in general by the California Forest Experiment Station,[3] and more specifically in a study of the total forest floor.[4]

It is only necessary to recall here that the slopes are steep, from 10° to 50°, and that the chaparral in Bell Canyon, at elevations between 2,500 and 3,500 feet, has come in since the severe fire of 1919, whereas that in Fern Canyon, at elevations from 4,500 to 5,400 feet, has been undisturbed for more than 50 years. The annual accumulations have been analyzed according to communities characterized by the predominating species of chaparral. Nine types are represented in Bell Canyon and 10 in Fern.

The collections were made by placing trays 3 feet square, with wooden edges 2 inches high, and 40-mesh screen wire for the bottoms, alternately 2 rods above and below the contour trails at the representative points where the rain gages were located by the Forest Experiment Station. The 64 trays in Bell Canyon and 54 in Fern may thus be considered to be representative of the variations in the vegetation in the two canyons, and perhaps by inference over a wider area. The material in the trays has been collected in late April in each of the 3 years. For various reasons, collections were not made from all the trays each year, so that the actual records comprise less than three times the number of trays.

Frequently there was evidence that organic and mineral material had entered the trays from above by creep or washing, or that some material had been lost at the lower edge. All such samples were excluded from the analysis. However, even when no creep was evident, the material often contained some mineral matter, and consequently all of the samples were separated from any mineral matter by a process of sieving and floating in the laboratory. The oven-dry

[1] Received for publication May 25, 1938.

[2] The work was made possible by the allotment of funds from a special State appropriation for research in forestry in cooperation with Federal agencies, and has been carried on with the cordial cooperation of the California Forest Experiment Station.

[3] UNITED STATES FOREST SERVICE, CALIFORNIA FOREST AND RANGE EXPERIMENT STATION. THE SAN DIMAS EXPERIMENTAL FOREST. 19 pp., illus. Berkeley, Calif. Revised, 1936.

[4] KITTREDGE, JOSEPH, JR. THE FOREST FLOOR OF THE CHAPPARAL IN SAN GABRIEL MOUNTAINS, CALIF. Jour. Agr. Research 58: 521-535, illus. 1939.

weights were then determined and converted to metric tons per acre. An attempt was made with the 1935 collections to separate the leaf material from the rest of the accumulations in order to learn what percentage the leaves made of the total and also their amount in dry weight per acre.[5] These data, the relations between the individual years, and the ratio of the annual accumulation to the total forest floor are shown in table 1.

TABLE 1.—*Annual accumulation of litter, in chaparral in Bell and Fern Canyons, 1935–37*

BELL CANYON

Chaparral type [1]	Average annual accumulation per acre [2]	Samples	Each year as percentage of 3-year average			Average total floor per acre [2]	Ratio of total to annual accumulation	Leaves only		
			1935	1936	1937			Samples	Amount per acre [2]	Percentage of 1935 annual accumulation
	Metric tons	*Number*	*Percent*	*Percent*	*Percent*	*Metric tons*		*Number*	*Metric tons*	*Percent*
Ag	1.85	2	88	112	-------	4.13	2.2	1	1.08	66.7
Cer–Af	.79	23	80	163	100	5.17	6.5	12	.29	47.9
Af–Cer	.76	27	64	154	84	4.21	5.5	8	.28	49.4
Sm–Cer	.67	7	72	170	57	7.40	11.0	2	.29	52.5
Qd–Cer	.63	12	68	155	97	3.75	6.0	3	.17	54.1
Cer–Sm	.54	13	80	146	120	6.72	12.4	4	.30	62.0
Gv	.49	5	43	133	155	9.50	19.4	1	.20	54.6
Af–Sm	.37	13	59	130	93	4.82	13.0	3	.01	5.6
Sm–Eff	.34	8	94	77	126	2.92	8.6	3	.19	61.0
All types	.64	110	71	145	[3] 91	5.31	8.4	37	.27	53.0

FERN CANYON

Ag–Af	1.40	3	65	124	110	20.56	14.7	1	0.40	44.5
Ag–Cd	1.08	4	88	104	102	20.16	18.7	1	.41	43.1
Ag	.98	13	81	116	108	21.18	21.6	4	.51	44.6
Qw–Ag	.90	2	84	-------	117	17.57	19.5	2	.27	39.0
Qc–Qwf	.60	9	73	122	107	12.84	21.4	2	.30	56.6
Qwf	.53	8	57	113	96	12.29	23.2	4	.22	43.5
Cd–Ag	.48	5	69	98	133	19.74	41.1	2	.16	47.7
Qc	.42	56	95	127	98	12.24	29.1	14	.16	44.9
Pm	.20	3	100	115	80	10.11	50.5	1	.07	36.9
Af	.19	3	69	126	111	4.69	24.7	1	.001	.7
All types	.57	106	83	[4] 116	100	15.06	26.5	32	.23	44.4

[1] Abbreviations here used: Af, *Adenostoma fasciculatum*, chamiso; Ag, *Arctostaphylos glauca*, manzanita; Qc, *Quercus chrysolepis*, canyon live oak; Ccr, *Ceanothus crassifolius*; Cd, *C. divaricatus*; Eff, *Eriogonum fasciculatum* var. *foliolosum*, wild buckwheat; Gv, *Garrya veatchii*, silktassel; Qd, *Quercus dumosa*, scrub oak; Qwf, *Q. wislizenii* var. *frutescens*, interior liveoak; Sm, *Salvia mellifera*, black sage; Pm, *Pseudotsuga macrocarpa*, bigcone-spruce.

[2] Metric tons oven-dry weight after separation of mineral matter.

[3] Based on 8 types.

[4] Based on 9 types.

The average annual accumulation for the different chaparral types varied from 0.19 metric ton per acre for chamiso (Af) up to 1.85 for manzanita (Ag). In a general way, those types that contained manzanita or *Ceanothus* tended to have heavier annual accumulations than the types characterized by chamiso black sage (Sm), false buckwheat (Eff), oak (Qd, Qwf, or Qc), or bigcone-spruce (Pm). The types in the table are arranged in descending order of the average annual

[5] The help of J. E. Aschoff, W. E. Davis, E. D. Marshall, and J. Vlamis in this work is gratefully acknowledged.

accumulation for each canyon. It is at once evident that the differences between adjacent types are not large, although the highest is three to five times the lowest. When all of the types for each canyon are combined in a single average, the annual accumulation for Bell Canyon was 0.64 and for Fern Canyon 0.57 metric ton per acre.

The amount of the annual accumulation varied from year to year for the same sampling points. This might be expected from the well-known variation between seasons as to their favorableness for the growth and development of the vegetation. In the case of the evergreen species, however, it is not the previous season but one of several years earlier in which the reason for a light or heavy fall must be sought. With few exceptions, all of the types in each canyon showed the lowest accumulation in 1935, the highest in 1936, and intermediate amounts in 1937. These figures, expressed as percentages of each year to the 3-year averages, are shown in the table for each of the chaparral types. In individual communities the spread between the different years may be considerable, as for example in the chamiso-*Ceanothus* (Af–Ccr) in Bell Canyon, where the 1935 accumulation constituted 64 percent, the 1936, 154 percent, and the 1937, 84 percent of the 3-year average for the type. Combining all types for Bell Canyon, the average percentages were 71, 145, and 91; and for Fern Canyon 83, 116, and 100 for 1935, 1936, and 1937 respectively. Both for the individual types and for the averages, the variation between years was less marked in Fern Canyon than in Bell Canyon.

When the figures for the annual accumulation are divided into those for the total forest floor, a series of ratios is obtained which represents the relation between the total and the annual accumulations. The sampling points for total forest floor were not identical with those for the annual accumulations, although both were mechanically spaced over the same area. For that reason, the ratios are more reliable for the types with larger numbers of samples than for those with fewer samples. These ratios also indicate the number of years of annual accumulation which would be required to yield the totals if accumulation were the only process involved. Of course, actually the processes of decomposition are going on in the forest floor each year, so that the ratio of total to annual should be less than the number of years since the last severe fire. In general this was the case. The ratios for Bell Canyon ranged from 2.2 to 19.4, with an average of 8.4 where the San Gabriel fire occurred from 16 to 18 years prior to the years of collection. In Fern Canyon, on the other hand, the ratios ranged from 14.7 to 50.5 with an average of 26.5 where the vegetation had been undisturbed for more than 50 years. The relation between this ratio and either the total forest floor or the annual accumulation alone is not well defined in Bell Canyon; but in Fern, the types with high accumulation and larger amounts of forest floor, namely, those characterized by manzanita (Ag), tend to have low ratios, whereas the canyon live oak (Qc) type has a rather high ratio. Because of the many factors that influence the rate of decomposition of the forest floor, it is readily apparent that this ratio, in which rate of decomposition is an important factor, may vary widely with types of vegetation, or with small differences in site.

The annual accumulation of leaf material expressed as a percentage of the total accumulation, and in metric tons per acre, is given for the 1935 season. In Bell Canyon the leaves constituted from 47.9 to

66.7 percent of the annual accumulation, with one exception. The average was 53 percent. In Fern Canyon with one exception the percentages ran a little lower, from 36.9 to 56.6 percent, with an average of 44.4 percent. In both canyons the types characterized by chamiso (Af) formed the exceptions, with percentages of 0.7 and 5.6.

The dry weights per acre of leaf material ranged from less than 0.01 to more than 1.00 metric ton per acre, with an average of 0.27 for Bell Canyon and 0.23 for Fern Canyon. Again the chamiso type forms exceptions, with very low weights of leaf material. Among the other types, the only noticeable tendency is for the heavier amounts of leaf material to be associated with the types containing manzanita (Ag). These are also the types which tend to have the heavier amounts of forest floor.

In both the total annual accumulation and the dry weight of leaf material, the averages for the two canyons are surprisingly close, considering the greater size and apparent luxuriance of the long-undisturbed types of Fern Canyon.

The collections which have been excluded in the foregoing analysis, because of creep of mineral and other material from above over the 2-inch upper side of the tray, provide incidentally an indication of the prevalence of this kind of soil movement on the slopes. When the trays are placed, the upper edge constitutes a barrier to creep, 2 inches or a little more in height above the soil surface, and roughly perpendicular to the slope. When material fills the resulting wedge of space above the tray to such an extent that some of it spills over into the tray, an appreciable movement of soil is indicated. The trays in Bell and in Fern Canyons for each of the years 1935, 1936, 1937, provided a record of the prevalence of creep of this magnitude on these two watersheds. Because the trays were mechanically distributed, the proportion of them which were affected by creep should be roughly indicative of the proportion of area which was subject to soil movement of similar character and intensity. However, in making this application to area, a correction is necessary because the creep is effective over only a part of the 3-foot length of the upper edge of the tray. Measurements of the actual distance subject to creep made first in 1938, indicate for Bell Canyon, an average of 1.2 feet, or 40 percent of the 3-foot length. For the 3 years in Bell Canyon with 164 records as a basis, 23 percent of the trays showed considerable creep, 18 percent showed a little, and the remaining 59 percent none. In other words, the creep in Bell Canyon each year is sufficient to override the 2-inch barrier of 41 percent of the trays. Applying the correction of 40 percent of the length, 16 percent of the area is probably subject to creep.

In Fern Canyon there was marked creep in 17 percent of the trays, a little in 13 percent, and none in the remaining 70 percent, on the basis of 150 records. The actual creep covers 1.8 out of 3 feet, or 60 percent on an average. Then, in this canyon with the older undisturbed vegetation, creep is indicated for 30 percent of the trays, and for 60 percent times 30, or 18 percent of the area, as compared with 16 percent in Bell Canyon.

The occurrence of creep was analyzed in more detail in relation to the degree of slope. In Bell Canyon the trays in which creep was recorded had the same average slope—35°—as those showing no creep. In Fern Canyon, on the other hand, the marked creep occurred

in trays on an average slope of 36°, a slight creep in those of 33°, and no creep in those of 28°. In general, it seems to be the location of individual trays with respect to local peculiarities of surface or of animal activity, rather than the systematic variation in degree of slope that affects the creep.

SUMMARY

The results of the study may be summarized in the following statements.

The average annual accumulation of organic material in the chaparral communities of diverse composition, age, and location rarely varied beyond the amounts of 0.2 to 1.4 metric tons per acre, with a general average of 0.6.

In different years, the annual accumulation varied from less than 50 to over 150 percent of the 3-year average.

The ratio of total forest floor to the annual accumulation increased with age of the vegetation from about 8 in the 18-year-old chaparral of Bell Canyon to over 26 in the more than 50-year-old types of Fern Canyon.

Leaf material constituted from 37 to 67 percent of the annual accumulation and amounted to about 0.25 metric ton per acre on an average. Two exceptions, with percentages of 0.7 and 5.6, may be mentioned for types to which chamiso gave character.

Sufficient creep of loose surface material took place annually to override a 2-inch barrier on 18 percent of the area of Fern Canyon and on 16 percent of the area of Bell Canyon.

138256—39——5

A FURTHER STUDY OF THE PRODUCTION OF MILK OF ABNORMAL COMPOSITION BY COWS FREE FROM UDDER STREPTOCOCCI[1]

By E. G. HASTINGS, *chairman, Department of Agricultural Bacteriology,* and B. A. BEACH, *chairman, Department of Veterinary Science, Wisconsin Agricultural Experiment Station*

INTRODUCTION

The results of a study of the composition of the first 30 cc of milk drawn from each quarter of the udders of 31 cows during their first lactation period were presented in an earlier report.[2] In some of the animals the milk conformed so closely to accepted standards for chlorine content and for pH and catalase values as to indicate that the mammary glands were normal in all respects. In other animals the deviations from the usual standards were so marked and persisted so long as to indicate an abnormal condition in one or more quarters of the gland, and in still others the deviations were so slight or so inconstant as to make difficult the assignment of the animal to a normal or an abnormal group. The deviations in composition were such as would be expected when some part of the gland was inflamed. The foremilks were more alkaline, lower in titratable acidity, slower to coagulate with rennet, and higher in catalase and chlorine than foremilk that is considered normal.

Since bacteria and especially certain streptococci are believed responsible for inflammation of the udder, much effort was made to obtain a quantitative and qualitative picture of the micro-organisms in the separate quarters of each animal throughout the lactation period. For this purpose various culture methods were used.

The findings did not conform to expectation in the case of the animals whose mammary glands showed evidences of inflammation at some level of intensity, for no streptococci were found and the abnormalities in the composition of the milk could be related to no definite qualitative or quantitative deviation in the flora of the udder. Nevertheless if the deviation of the milk from recognized standards is accepted as evidence of inflammation of the udder, a large part of the group would have been considered unfit for the production of milk for human consumption.

Some of the animals were retained in the herd during the second lactation period and observations similar to those made in the first period were continued. The results are presented in the present paper.

EXPERIMENTAL PROCEDURE

The term "normal" refers to an animal whose foremilk from each quarter conformed to two of the following criteria: A pH value below 6.8, a catalase value below 50, and a chlorine content below 0.15 percent. The methods of analyses were described in the previous

[1] Received for publication July 29, 1938.

[2] HASTINGS, E. G., and BEACH, B. A. THE PRODUCTION OF MILK OF ABNORMAL COMPOSITION BY ANIMALS FREE FROM UDDER STREPTOCOCCI. Jour. Agr. Research 54: 199-220., 1937.

paper. The use of more than one criterion is necessary since animals vary and it is possible to have the milk of a normal animal fail to conform to one of the above criteria, but probably not to two except in the colostric period or very late in the lactation period. The criteria for chlorine and pH are probably reasonably correct; that for catalase is undoubtedly too high since the milk of many animals does not at any time reach this value. It is believed that the above criteria when applied to a considerable number of samples taken at intervals throughout a lactation period offer a reasonably safe means of grouping animals on the basis of condition of the udder. It seems probable that nearly all animals will show abnormalities in the milk from time to time, and that such transitory deviations from the usual condition are of little importance.

Twelve of the thirty-one animals, whose records were presented in the previous report were studied in their second lactation period and also one animal not included in the earlier experiments. For purposes of discussion, the 13 animals are placed in four groups as follows:

Group 1. Cows 1, 7, and 8, which were normal in both the first and second period.

Group 2. Cow 6, which was normal in the first and abnormal in the second period.

Group 3. Cows 22 and 32, which were questionably normal in the first and abnormal in the second period.

Group 4. Cows 5, 11, 15, 21, 31, 34, and 39, which were abnormal in each period.

The herd was maintained for the purpose of studying certain phases of pasture management, and the animals to be retained in the herd during the second period were selected primarily with regard to time of freshening, the condition of the udder noted in the first period being a secondary consideration. This explains why a much larger number of the animals classed as abnormal in the first period were retained than of those classed as normal.

The usual sample consisted of the first 30 cc of milk from each quarter. At less frequent intervals the remainder of the milk produced by each quarter was examined in greater detail. The former are referred to as "routine," the latter as "special" samples.

The record of the animals normal in each period is presented for both the first and the second periods, but for the remainder only such reference is made to the first period as is necessary to give the reader a reasonably complete picture of the animal during the entire period of observation. The record of the normal animals serves as a standard for comparison.

ANIMALS NORMAL IN THE FIRST AND SECOND LACTATION PERIODS

Cow 1 was observed during 245 days in the first lactation period, in which 17 routine samples were taken from each quarter, and during 254 days in the second period, in which 9 routine samples were secured from each quarter. In each period the last sampling was made about 1 month before the animal was dried off. The data of table 1 present the picture of a cow producing milk that conforms closely to accepted standards. The normality of the animal was reflected by the normal

condition of the udder at slaughter, 1 month after the last observation was made.

Cow 7 was observed 285 days in the first period, in which 30 routine samples were collected from each quarter, and in 302 days of the second lactation period, in which 10 samples were collected. The final sampling was made about 1 month before the end of each period. The detailed data in table 1 are those of a normal animal. The maximum percent of chlorine, 0.193, may attract attention. Since the values noted 7 days before the one on which the maximum value was found and the one 7 days thereafter were normal, and since there was no disturbance in the catalase or in the reaction of the milk, this maximum value is considered to have no significance.

Cow 8 presents a picture of normality in the case of the 32 samples taken from each quarter in the first 322 days of the first period and the 8 samples in the 274 days of the second. About 12 pounds of milk were produced per day at the date of last sampling in the first period, about 1 month before drying off. Table 1 shows the values for chlorine and catalase to be normal. The maximum values for chlorine, 0.211, and for catalase, 58, are not significant for the reasons presented in the discussion of cow 7. The data for the samples taken 1 month before the end of the second period are not presented. They were abnormal with respect to chlorine and catalase. Thus the chlorine content of the milk from the quarters was 0.276, 0.224, 0.252, and 0.248 percent, respectively. The daily production at that time was 12 pounds per day. It seems certain that in the first period there was no particular change in the composition of the milk as the end of the lactation period approached, but at the end of the second period, marked changes occurred. It is thus evident that an animal reacts differently in different periods as regards the change in the composition of the milk as production decreases.

TABLE 1.—*Percentage of chlorine and of catalase in the milk of cows giving normal milk in the first and second lactation periods*

Cow and quarter	First lactation period				Second lactation period			
	Minimum chlorine	Maximum chlorine	Minimum catalase	Maximum catalase	Minimum chlorine	Maximum chlorine	Minimum catalase	Maximum catalase
Cow No. 1:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0.100	0.155	2	29	0.117	0.161	4	49
2	.107	.144	2	22	.111	.134	3	20
3	.111	.141	5	28	.111	.127	3	32
4	.107	.158	5	31	.100	.141	5	35
Cow No. 7:								
1	.117	.193	0	20	.104	.138	5	18
2	.104	.141	0	20	.104	.138	5	24
3	.121	.144	10	75	.100	.127	5	26
4	.111	.141	0	31	.104	.138	3	58
Cow No. 8:								
1	.134	.182	0	16	.111	.185	3	22
2	.127	.148	0	22	.117	.211	6	58
3	.127	.155	0	22	.121	.175	4	36
4	.124	.161	0	28	.111	.178	5	23

One set of samples obtained early in the third period was normal in composition.

AN ANIMAL NORMAL IN THE FIRST LACTATION PERIOD AND BECOMING ABNORMAL IN THE SECOND

The data for the first lactation period of cow 6 are presented in table 2. The period of observation was 307 days in length and 33 samplings were made. At one sampling the chlorine content of each quarter was high, being 0.217, 0.182, 0.255, and 0.178 percent, respectively, for the quarters. The chlorine values 7 days earlier and 7 days later were normal, as were the catalase values on the specific date. The abnormal values were, therefore, either erroneous or indicative of a transitory condition and were not included in table 2.

TABLE 2.—*Percentage of chlorine and catalase in the milk of cow No. 6 which gave normal milk in the first but abnormal in the second lactation period*

Quarter	First lactation period				Second lactation period			
	Minimum chlorine	Maximum chlorine	Minimum catalase	Maximum catalase	Minimum chlorine	Maximum chlorine	Minimum catalase	Maximum catalase
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0.124	0.175	0	36	0.111	0.193	6	90
2	.117	.148	0	34	.107	.303	5	254
3	.121	.144	6	41	.104	.155	6	66
4	.121	.144	3	48	.107	.172	4	54

Ten sets of samples were obtained during 302 days of the second period. The data in table 3 are presented to show the gradual increase in abnormality in the last third of the second period. Each quarter is involved in a change for which no reason was evident.

TABLE 3.—*Percentage of chlorine and catalase in the milk of cow No. 6 on certain days in the last third of the second lactation period*

Days since freshening	Quarter 1		Quarter 2		Quarter 3		Quarter 4	
	Chlorine	Catalase	Chlorine	Catalase	Chlorine	Catalase	Chlorine	Catalase
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
203	0.193	90	0.127	14	0.131	20	0.148	18
237	.155	27	.185	62	.138	19	.138	22
274	.158	54	.224	254	.127	66	.148	54
302	.189	80	.303	190	.155	40	.172	36

ANIMALS QUESTIONABLY NORMAL IN THE FIRST LACTATION PERIOD AND ABNORMAL IN THE SECOND

In the case of cow 32 the abnormality during the first period was confined to quarter 2. Of the 16 samples taken from the one hundred and fifth to the three hundred and ninth day, 4 were abnormal and the remainder differed sufficiently from those collected earlier in the period to indicate that a decided change in the quarter had occurred. The data for the routine samples are shown in table 4, and for the special samples of the second period in table 5. It is to be noted that quarter 3 was normal in each period and that as the second period passed, the percentage of milk from this quarter constantly increased. The animal produced 260 pounds less milk in the same number of days (243) in the second period than in the first. It seems probable that

the gradually increasing abnormality in the other quarters was responsible for the decrease in the production of these quarters.

TABLE 4.—*Percentage of chlorine and of catalase in the milk of cow No. 32 which gave questionably normal milk in the first and abnormal in the second lactation period*

Quarter	First lactation period					Second lactation period				
	Minimum chlorine	Maximum chlorine	Average chlorine	Minimum catalase	Maximum catalase	Minimum chlorine	Maximum chlorine	Average chlorine	Minimum catalase	Maximum catalase
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1	0.114	0.144	0.125	0	57	0.138	0.168	0.145	16	264
2	.114	.239	.172	10	233	.131	.185	.161	16	230
3	.111	.155	.124	0	49	.107	.165	.121	9	78
4	.114	.144	.133	4	108	.097	.189	.152	9	400

TABLE 5.—*Yield, catalase, and chlorine content, and acidity of both the foremilk and the remainder of the milk (whole milk) from the different quarters of cows giving abnormal milk, sampled at various times after lactation began*

COW NO. 32

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
37	1	4.7	19	196	99	0.161	0.127	0.117	0.144
	2	7.2	27	296	306	.155	.138	.126	.162
	3	7.2	27	31	19	.114	.097	.162	.162
	4	7.0	27	34	19	.104	.100	.180	.189
Total		26.1							
85	1	2.4	14	32	12	.138	.093		
	2	4.1	25	50	47	.138	.117		
	3	5.2	32	44	25	.131	.107		
	4	4.6	29	5	3	.097	.100		
Total		16.3							
163	1	2.0	15	44	16	.200	.161	.153	.184
	2	3.5	25	61	65	.197	.168	.153	.180
	3	4.7	34	6	8	.155	.138	.180	.189
	4	3.6	26	220	224	.197	.178	.180	.171
Total		13.8							
218	1	1.0	15	33	36	.144	.114	.162	.189
	2	1.0	15	79	28	.152	.127	.153	.180
	3	2.8	42	19	18	.117	.104	.180	.198
	4	1.8	28	228	255	.185	.141	.126	.171
Total		6.6							

COW NO. 22, FIRST LACTATION PERIOD

153	1	2.0	21	10	21	0.117	0.111	0.153	0.157
	2	3.1	33	15	7	.124	.114	.162	.157
	3	2.6	28	10	4	.121	.117	.153	.153
	4	1.7	18	76	38	.138	.138	.153	.157
Total		9.4							
161	1			13	13	.152	.127	.130	.162
	2			14	22	.165	.155		.162
	3			17	22	.148	.117		.153
	4			56	46	.178	.131	.108	.153
183	1	3.1	19	74		.178		.108	
	2	4.8	30	10	16	.144	.131	.126	.153
	3	5.3	33	5	6	.127	.114	.144	.162
	4	2.8	18	20	26	.152	.127	.126	.144
Total		16.0							

TABLE 5.—*Yield, catalase, and chlorine content and acidity of both the foremilk and the remainder of the milk (whole milk) from the different quarters of cows giving abnormal milk, sampled at various times after lactation began*—Continued

COW NO. 22, FIRST LACTATION PERIOD—Continued

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
219	1	3.2	18	34	27	.155	.124	.153	.162
	2	5.7	32	70	29	.134	.134		.162
	3	6.1	34	17	10	.131	.117	.153	.180
	4	2.9	16	70	33	.144	.127	.153	.162
Total		17.9							

COW NO. 22, SECOND LACTATION PERIOD

6	1	5.2	23	200	126	0.214	0.138	0.099	0.171
	2	8.5	39	210	107	.214	.131	.081	.189
	3	2.0	29	264	242	.276	.155	.108	.171
	4	6.5	29	47	45	.148	.121	.144	.189
Total		22.2							
39	1	7.0	26	23	18	.144	.117	.126	.153
	2	10.2	38	14	7	.127	.111	.144	.172
	3	3.2	12	188	82	.221	.141	.063	.126
	4	6.2	24	8	11	.111	.107	.162	.162
Total		26.6							
83	1	5.0	24	27	19	.155	.124	.108	.144
	2	8.1	39	18	12	.148	.117	.117	.144
	3	2.1	10	70	22	.172	.117	.104	.135
	4	5.6	27	5	9	.121	.114	.139	.153
Total		20.8							
80	1	2.4	22	240	214	.296	.158	.045	.130
	2	5.2	47	21	12	.148	.114	.121	.162
	3	.7	6	85	117	.200	.117	.090	.153
	4	2.7	25	24	24	.161	.117	.108	.153
Total		11.0							
213	1	2.4	16	198	120	.255	.138	.072	.144
	2	7.2	49	35	45	.148	.117	.121	.153
	3	1.2	8	88	48	.200	.121	.081	.153
	4	3.9	27	36	20	.152	.111	.126	.153
Total		14.7							

COW NO. 5, SECOND LACTATION PERIOD

27	1	3.6	19	4	7	0.134	0.131	0.135	0.144
	2	5.5	30	13	9	.148	.131		.144
	3	4.8	26	30	30	.158	.134	.117	.126
	4	4.7	25	16	8	.127	.131	.139	.130
Total		18.6							
31	1	5.1	24	9	20	.127	.124	.144	.135
	2	5.6	26	13	16	.138	.117	.126	.135
	3	5.5	25	13	12	.134	.114	.117	.135
	4	5.5	25	7	12	.124	.117	.126	.135
Total		21.7							
96	1	3.2	22	238	42	.197	.144	.108	.122
	2	3.7	25	140	51	.217	.155	.099	.126
	3	4.0	28	150	37	.203	.155	.090	.126
	4	3.7	25	64	27	.182	.138	.108	.135
Total		14.6							
231	1	3.7	25	85	22	.152	.148	.135	.135
	2	3.8	26	83	18	.152	.144	.144	.135
	3	3.8	26	107	22	.144	.141	.144	.144
	4	3.6	23	51	13	.144	.138	.144	.135
Total		14.9							

TABLE 5.—*Yield, catalase, and chlorine content, and acidity of both the foremilk and the remainder of the milk (whole milk) from the different quarters of cows giving abnormal milk, sampled at various times after lactation began*—Continued

COW NO. 11, SECOND LACTATION PERIOD

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
31	1	5.5	23	22	53	0.141	0.144	0.144	0.144
	2	.5	2	290	261	.303	.211	.054	.090
	3	10.9	46	3	5	.144	.138	.162	.162
	4	6.6	29	6	5	.141	.141	.162	.153
Total		23.5							
75	1	4.6	25	54	146	.165	.152	.135	.126
	2	.4	2	198	196	.248	.211	.063	.090
	3	7.9	43	5	7	.148	.144	.127	.126
	4	5.6	30	4	8	.148	.141	.135	.126
Total		18.5							
144	1	4.4	31	101	102	.203	.182	.099	.126
	2	.4	3	183	98	.293	.231	.054	.072
	3	7.6	55	16	14	.168	.152	.135	.144
	4	1.6	11	96	45	.185	.175	.117	.117
Total		14.0							
171	1	3.0	29	135	99	.221	.158	.081	.121
	2	.3	3	162	216	.262	.224	.063	.090
	3	6.2	59	16	16	.158	.138	.135	.144
	4	1.0	9	79	58	.189	.158	.099	.126
Total		10.5							
185	1	1.5	24	128	81	.237	.149		
	2	.1	2	158	187	.301	.237		
	3	4.0	65	14	19	.165	.145		
	4	.5	9		59	.207	.166		
Total		6.1							

COW NO. 15, SECOND LACTATION PERIOD

28	1	6.8	19	31	11	0.172	0.158	0.117	0.144
	2	10.7	30	13	6	.189	.158	.112	.135
	3	11.7	33	19	30	.175	.172	.126	.126
	4	6.5	18	4	26	.158	.158	.148	.144
Total		35.7							
64	1	4.5	19	66	24	.214	.172	.099	.117
	2	8.1	33	6	3	.211	.178	.090	.117
	3	7.4	30	14	13	.207	.172	.108	.108
	4	4.2	18	10	5	.182	.168	.135	.126
Total		24.2							
161	1	2.8	15	191	189	.248	.203		
	2	6.2	32	67	100	.200	.197		
	3	6.3	33	41	75	.182	.203		
	4	3.7	20	59	77	.175	.193		
Total		19.0							

COW NO. 21, SECOND LACTATION PERIOD

13	1	7.1	27	11	13	0.127	0.141	0.180	0.171
	2	6.8	25	74	157	.158	.148	.144	.162
	3	7.4	28	18	42	.134	.131	.180	.171
	4	5.4	20	19	59	.148	.127	.171	.162
Total		26.7							

TABLE 5.—*Yield, catalase, and chlorine content, and acidity of both the foremilk and the remainder of the milk (whole milk) from the different quarters of cows giving abnormal milk, sampled at various times after lactation began*—Continued

COW NO. 21, SECOND LACTATION PERIOD—Continued

Days since freshening	Quarter	Yield		Catalase		Chlorine		Acidity	
				Foremilk	Whole milk	Foremilk	Whole milk	Foremilk	Whole milk
		Pounds	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
54	1	8.5	34	4	7	0.121	0.111	0.153	0.162
	2	4.1	16	137	199	.182	.175	.117	.117
	3	6.2	24	6	9	.117	.121	.162	.162
	4	6.6	26	19	47	.117	.124	.166	.157
Total		25.4							
78	1	7.9	32	3	31	.121	.114	.162	.162
	2	4.3	17	238	146	.282	.161	.054	.135
	3	6.5	26	32	33	.189	.131	.099	.139
	4	6.2	25	10	16	.127	.117	.130	.162
Total		24.9							
118	1	4.1	30	12	11	.161	.131	.117	.135
	2	2.7	19	227	145	.307	.165	.054	.108
	3	4.0	29	20	32	.138	.144	.135	.126
	4	3.0	22	11	22	.127	.134	.135	.135
Total		13.8							
177	1	4.6	32	20	12	.117	.121	.135	.144
	2	2.3	16	122	108	.158	.155	.117	.126
	3	3.5	24	113	45	.168	.152	.117	.121
	4	3.9	28	39	20	.141	.127	.135	.135
Total		14.3							
253	1	3.7	33	30	26	.175	.127	.108	.144
	2	2.2	19	204	96	.234	.138	.081	.135
	3	2.5	22	49	27	.193	.134	.108	.144
	4	3.0	26	18	21	.138	.131		.144
Total		11.4							

COW NO. 31, SECOND LACTATION PERIOD

41	1	7.2	28	15	23	0.111	0.104	0.189	0.171
	2	.0	0	282		.354			
	3	10.1	38	14	18	.114	.111	.162	.180
	4	9.0	34	31	6	.104	.104	.180	.171
Total		26.3							
77	1	6.9	29	7	5	.114	.111	.135	.162
	2	.0	0	258		.351		.045	
	3	10.0	43	5	6	.117	.114	.144	.162
	4	6.6	28	12	15	.117	.121	.144	.162
Total		23.5							
115	1	7.0	34	30	29	.127	.117	.135	.144
	2			233		.344		.036	
	3	6.3	32	18	26	.131	.121	.126	.135
	4	7.0	34	14	78	.127	.138	.144	.126
Total		20.3							
202	1	5.0	37	50	37	.231	.182	.126	.153
	2	.0	0	222		.397		.063	
	3	5.0	37	136	133	.252	.197	.108	.162
	4	3.4	26	234	224	.334	.231	.072	.139
Total		13.4							
257	1	2.3	39	106	78	.241	.161	.180	.126
	2	.1	1	227		.238	.217	.099	.081
	3	2.3	39	80	123	.234	.168	.081	.135
	4	1.2	21	266	224	.293	.172	.063	.135
Total		5.9							

Cow 22 showed no abnormalities during the first 3 months of the first period, but soon thereafter quarter 4 began to show abnormalities, which continued to the end of the period. Quarter 1 began to show deviations from the normal at a later date. The general nature of the changes is shown in table 5. It should be noted that the changes in the milk were confined to the foremilk; the entire milk from quarters 1 and 4 was not materially different from that of 2 and 3. The yield of the abnormal quarters was maintained as well as that of the normal quarters, and indeed the production of the animal was well maintained in the last part of the period, as the following figures show: Fourth month, 1,058 pounds; fifth month, 982 pounds; sixth month, 824 pounds; seventh month, 832 pounds; and eighth month, 828 pounds. The number of bacteria in the foremilk of quarter 4 was greater in the last part of the period than in the first. The samples from quarter 1, taken during the last 2 months, contained a streptococcus which from a limited study was judged to be of a type not generally believed to be concerned in the production of chronic mastitis.

The dry period was about 2 months in length. Quarter 1 was, in the second period, at first abnormal, then normal, and still later abnormal, while quarter 4 was quite normal during the entire second period. Quarter 3, which had been consistently normal in the first period, was consistently abnormal in the second. Quarter 2 was normal in each period. These changes are reflected in the changes in the relative percentages of the total yield produced by each quarter. In the first period, quarter 3 produced from 28 to 34 percent of the total milk; in the second from 6 to 12 percent. Quarter 4 yielded 16 to 18 percent and 24 to 29 percent in the respective periods. In the second period as in the first the entire milk from the abnormal quarters had much the same composition as that from the normal quarters, but a gradual deterioration of the animal is indicated by a decreased production of milk in the second period.

ANIMALS ABNORMAL IN BOTH THE FIRST AND SECOND LACTATION PERIODS

The remaining seven animals were abnormal in both periods. Cow 34 was observed for 315 days in the first period during which 31 samplings were made, and for 309 days in the second with 12 samplings. The animal was classed as abnormal during the last part of the first period, since the third quarter yielded from the one hundred and eleventh day to the end of the period milks which satisfied the criteria of abnormality. After a dry period of 3 months, the second period began with a normal condition in each quarter. After the two hundred and forty-fourth day, quarters 2 and 3 were definitely abnormal. It is evident from a study of all the data that quarters 2 and 3 began to change from their previous condition by the middle of the first period and did not return to that condition during the remainder of the period of observation.

It is probable that the chlorine content of the foremilk of this animal was about 0.12 to 0.14 percent. The percents of samples of foremilk exceeding these values in the first period were as follows:

Quarter:	*First period* Percent	Quarter:	*Second period* Percent
1	19	1	20
2	87	2	40
3	100	3	50
4	25	4	33

The case is thus one in which an abnormal condition was established gradually in the rear quarters at the middle of the first period and persisted throughout the second, becoming more marked in the latter part of the second. The productivity was not impaired since the animal produced 8,129 pounds of milk in 308 days of the second period as compared to 5,950 pounds in 311 days of the first period.

Cow 39 was apparently normal during the first 180 days of the observation period of 320 days in the first period. Thereafter quarters 3 and 4 yielded abnormal milk quite consistently to the end of the period. The dry period was about 6 weeks in length. For the first 55 days of the second period, the milk from all quarters was normal. From then on to the end of the period of observation at the two hundred and twenty-third day the milk of every quarter was abnormal.

Only foremilk samples were examined in the first period. The more detailed study of the second period showed that the abnormality was confined to the foremilk and that even in the first part of the second period, each quarter was abnormal in some degree. Five thousand four hundred and seventy-seven pounds of milk was produced in 244 days of the first period and 6,759 pounds in 244 days of the second. The increase in production indicates that the abnormality was of low intensity.

The record of the remaining cows, 5, 11, 15, 19, 21, and 31, during the first period was presented in the earlier paper. Only such data will be repeated as are necessary to relate the record of the first period to the second.

Cow 5 was abnormal throughout the first period. The relative condition of the quarters is shown by the percentage of abnormal samples:

Quarter:	*Percent*
1	15
2	32
3	61
4	3

The record for the second period is shown in table 5. Quarter 4 was relatively free from abnormality during each of the periods. The percentage of milk produced by this quarter ranged from 23 to 25. The low intensity of the abnormality is shown by the relative yields of the different quarters, and by the fact that the entire amount of milk produced by any quarter was rarely of abnormal composition. The composition of the foremilk, especially from quarter 3, would have indicated the removal of the animal even in the first period if indirect tests for inflammation had been relied on.

Cow 11 was observed for 224 days in the first period, which was marked by the consistent abnormality of quarter 2 from the very beginning and the consistent normality of the other three quarters. The animal was observed for 173 days in the second period (table 5). Quarter 2 was essentially nonfunctioning in the second period and quarter 4 became relatively less and less productive toward the end of the period. Quarter 3 was then producing 65 percent of the total yield. The animal was slaughtered soon after the last sampling was made. The post mortem revealed nothing of importance other than had been indicated by the milk.

Cow 15 was in a normal condition at the beginning of the first period, if one can rely on the catalase and pH values. During the

early part of the period chlorine was not determined quantitatively, except to learn whether it was above 0.15 percent. The subsequent findings indicated that the normal chlorine value was considerably above this level, for in no instance in the numerous quantitative observations made in each of the two periods was the percentage as low as 0.15, even when catalase and pH values were normal. Quarters 1, 2, and 3 became abnormal within less than 3 months in the first period and remained consistently so to the end of the period on the three hundred and sixth day. Quarter 4 was classed as normal. The dry period was 94 days in length. There was a period of normality at the beginning of the second period, as is shown by the findings on the twenty-eighth day (table 5). One should recognize that chlorine percentages of 0.18 are normal to this animal. By the sixty-fourth day abnormalities were evident and by the one hundred and sixty-first day they were more pronounced. In contrast to the condition in the animals previously discussed, the entire amount of milk produced by one quarter of this animal was abnormal. The later findings, not presented in detail, showed that the abnormality increased in severity as the milking period lengthened. The chlorine content of quarters 1, 2, and 3 was from 0.3 to 0.4 percent.

Cow 21 was abnormal during practically all the first period. The relative condition of the quarters is shown by the following:

Quarter:	*Percent of 30 samples abnormal*
1	3
2	80
3	13
4	20

The relative condition of the quarters was much the same in the second quarter as in the first, as is indicated by the following:

Quarter:	*Percent of 10 samples abnormal*
1	0
2	94
3	25
4	0

The detailed data of cow 21 are presented in table 5. The abnormality of the milk was confined largely to the foremilk so far as acidity and chlorine are concerned; the catalase value of the entire milk remained high.

The second and third quarters of cow 31 became abnormal at about the middle of the first period, which was 379 days long, and the abnormal condition continued to the end of the period. No essential differences were noted between the two quarters. However, in the second lactation (table 5), quarter 2 produced no milk and quarter 3 produced from 32 to 43 percent of the total yield, whereas in the latter part of the first period the percentage was from 13 to 21. This is one of the few instances in which there is reason to believe that improvement in the condition of a quarter occurred.

INFLUENCE OF THE ABNORMALITY ON MILK PRODUCTION

The production of each of the 13 animals in practically an equal number of days in the two lactation periods is presented in table 6. The results are such as might be expected. Each of the animals normal in both periods showed an increase comparable to the expected

increase of a second over a first lactation. The animals normal or questionably normal in the first period and abnormal in the second showed decreases in two of the three cases. The animals abnormal in each period gave the most variable results, ranging from a decrease from the production of the first period to an increase of 44 percent. The animals are too few to indicate other than the trend induced by the abnormality. If the 13 are divided into two groups, one group consisting of the animals normal in each period and the other of the remainder, the average increase in production for the first group is 2,151 pounds; for the second 1,053 pounds. Thus the abnormality is seen to have reduced the yield over 1,000 pounds in a lactation period.

TABLE 6.—*Milk-production records of the cows as related to normality or abnormality of the milk*

Cow number	Condition of milk		Yield		Increase (+) or decrease (−) in second period
	First period	Second period	First period	Second period	
			Pounds	*Pounds*	*Pounds*
1	Normal	Normal	7,889	9,991	+2,102
7	do	do	7,353	9,418	+2,065
8	do	do	6,549	8,837	+2,288
6	do	Abnormal	8,989	10,655	+1,666
22 [1]	do	do	10,512	8,902	−1,610
32 [1]	do	do	7,313	7,053	−260
5	Abnormal	do	7,028	10,127	+3,099
11	do	do	5,975	6,178	+203
15	do	do	7,249	8,241	+992
21	do	do	7,145	10,163	+3,018
31	do	do	3,933	3,900	−33
34	do	do	5,950	8,129	+2,179
39	do	do	5,477	6,759	+1,282

[1] Cows 22 and 32 questionably normal in the first period.

DISCUSSION

The record of this isolated herd supports the view that the streptococci causing chronic mastitis are specific inhabitants of the udder and not adventitious forms from some other habitat, such as the digestive tract, and it shows further that it is possible to keep a herd free from them. The production of milk of abnormal composition by one or more quarters over long periods of time means that some agency other than streptococci is responsible for the abnormality.

The animals showed no evidence of abnormality other than that revealed by the periodic examination of the milk. There were practically no swollen udders, and physical changes in the milk were seldom noticed. From the absence of these two conditions the herd would have been classed by the usual herd owner as free from mastitis. If the presence of streptococci is demanded as proof of chronic mastitis, the herd would again have been classed as mastitis-free, since but one animal was shown to harbor streptococci in the udder. In this case the organism was shown to be of a type not supposed to cause mastitis. It produced no disturbance and seemed to be confined to the milk cistern.

In the earlier report, it was stated that no consistent bacterial picture had been noted for the group of cows producing abnormal

milk, nor for any one animal. In many instances the number of bacteria was greater in the abnormal foremilk than in the normal foremilk from other quarters of the animal. The exceptions were so numerous, however, that it is not safe to conclude that there was a causal relation between the bacteria that grew in the milk or culture media and the condition of the udder. Instances of a long series of abnormal milk samples from a quarter and a consistently low bacterial content of the foremilk and of the entire milk drawn from the quarter were noted. Again, in a series of consistently abnormal milks, the bacteria might be now high, now low. It may be that the intermittency noted by Johns and Hastings [3] in which a rhythmic variation in the composition of the milk is produced by a rhythmic variation in the number of bacteria, a seesaw between the parasite and the host, existed but was not detectable in the examination since the bacterial and chemical analyses were made on the same sample. It is possible that the examination has not been of the proper nature to reveal significant bacteria, significant because of their number or because of their quality. In the course of the work, numerous media were used and the other factors of the environment were varied. Some of the samples were cultured in litmus milk and on glucose peptone beef-extract agar slopes. Others were plated on veal infusion agar containing horse blood and on glucose agar. The difference in results secured on many samples with the two plating media was so great quantitatively as to indicate that a fancied feeling of safety is easily obtained when only one medium is employed.

After a year's study subsequent to the period represented in the previous report, and with the advantage of the experience acquired, the writers cannot now make a more definite statement as to the cause of the physiological disturbance than was therein made. They can only state as before that no evidence has been secured to indicate that the condition was due to a transmissible agent. The number of animals involved is too small to supply other than suggestive evidence. It would seem that the cause was inherent in the animal, for the four cows that had a normal history in the first lactation period had a normal history in the second, and the nine that were abnormal in the first period were abnormal in the second. Likewise it would appear unwise to conclude, simply on the basis of abnormal chemical reactions of the milk, that a cow is diseased unless other evidence can be secured showing that pathogenic organisms are present in the milk, or that the cow actually has physically detectable symptoms of mastitis. The significance, from the herd owner's standpoint, of the physiological disturbance noted in the animals of this herd cannot be stated, since the number of animals observed was small, and the observations were not continued beyond the second period. The significance from the standpoint of the user of milk, individual or manufacturer, seems slight, since the composition of the total amount of milk produced at a milking was within ranges considered as normal.

The question whether the physiological disturbance studied provides a favorable environment for *Streptococcus agalactiae* was discussed in the previous report, and further observations have been made by exposing some of the animals described herein to infection with *S. agalactiae*. The tentative conclusion is that the environment

[3] JOHNS, C. K., and HASTINGS, E. G. CONCERNING THE USE OF INDIRECT BIOCHEMICAL TESTS FOR THE DIAGNOSIS OF CHRONIC CONTAGIOUS MASTITIS. Canad. Jour. Research 16 (D. Zool. Sci.): 6-14, illus. 1938.

provided in the abnormal quarters is more favorable for the establishment of streptococci than that provided by the normal gland.

SUMMARY

The observations reported in an earlier paper regarding the production of abnormal foremilk in the first lactation period by cows free from udder streptococci have been extended for some of these animals during a second lactation period. The continued study does not permit any definite statement as to the cause of the disturbance or the transmissibility of its cause.

The disturbance tends to continue from one lactation period to another in the same quarters. It also tends to decrease production and to shorten the production period. The milk apparently is rarely so changed in composition as to be of less value either as fluid milk or as a raw material for the various milk products.

U. S. GOVERNMENT PRINTING OFFICE: 1939